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VOL. 14, SEC. C. JANUARY, 1936 NUMBER 1

STUDIES ON THE NATURE OF RUST RESISTANCE IN WHEAT

VII. CHEMICAL ANALYSES OF HYBRID LINES OF WHEAT DIFFERING IN THEIR RUST REACTIONS[1]

BY J. ANSEL ANDERSON[2]

Abstract

Four lots of wheat, representing the four possible combinations of seedling and mature-plant reaction to rust, were grown in the field, and leaves were collected at the seedling stage and after heading. The material was dried, ground and analyzed for:— total ash; total nitrogen; fat; cold- and hot-water-soluble organic matter, ash, reducing compounds, reducing sugars and invert sugar; alcohol-soluble matter; reducing compounds (from hemicelluloses) and nitrogen liberated by hydrochloric acid; reducing compounds (from cellulose) and nitrogen liberated by sulphuric acid; and for ash, protein and lignin (by difference) in the remaining residue. The material was also subjected to quantitative extraction with the following solvents in series:— ligroin, ether, chloroform, ethyl acetate, acetone, and ethyl alcohol.

Small but significant differences in constitution were found between the wheat classes. There was no evidence that these differences in constitution were related to rust reaction.

Introduction

Physiological susceptibility and resistance to stem rust in wheat appear to depend upon a balance or lack of balance between the physiological processes of the host and those of the parasite. The complicated relations which exist between the various wheat varieties and physiologic forms of the fungus preclude the possibility of any simple explanation of resistance and susceptibility. It seems probable that variations in physiology exist amongst both the wheat varieties and the rust forms, and that it is the combinations and permutations of these that give rise to the complicated relations which exist between host and parasite. If this be true, no solution of the problem of the fundamental nature of rust resistance can be expected until adequate studies have been made of the physiological processes of a wide range of susceptible and resistant wheat varieties and of a large number of physiologic forms of the fungus.

The chemist can contribute to these studies by analyzing the host tissues. It is true that such analyses must be carried out on dead material, but it must be borne in mind that the dead plant is a partial record of the metabolic activities of the protoplasm. A study of the compounds present in the dead tissue, which are end products of the biochemical reactions which have taken place, provides one method of comparing the physiology of different plants.

[1] *Manuscript received October 30, 1935.*
Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, Canada. Parts I-VI in this series were published in this Journal, 1 : 5-35, 86-99, 1929; 4 : 134-147, 1931; 11 : 667-686, 1934.

[2] *Biologist, National Research Laboratories, Ottawa, Canada.*

For many years, chemical investigations of the nature of rust resistance were unattractive since they were almost foredoomed to failure because of the nature of the material available for analysis. In order to obtain a series of wheat varieties differing widely in their reactions to stem rust, it was necessary to select representatives of several species of wheat. Resistant varieties were taken from the emmer group and susceptible varieties from the *vulgare* group. The interpretation of the results of analyses was thus complicated by the fact that such differences as might be found might easily represent morphological or physiological characters associated with the class of wheat rather than with rust reactions.

During the past few years plant breeders working on the development of new rust-resistant wheat varieties have produced material from which the chemist can select far more suitable series of wheats for his investigations. Such a series was obtained about three years ago from the Dominion Rust Research Laboratory, Winnipeg. It consists of twelve hybrid lines from a Marquis × H-44-24 cross which may be grouped into four classes representing the four possible combinations of seedling and mature-plant reaction to stem rust. These lines are homozygous for rust reaction but with respect to morphological characters represent random selections in the F_2 generation. In the F_3 generation they were found to be true breeding for rust resistance, and since then have been carried along in bulk without re-selection of any kind. They are thus pure lines for rust resistance only and for morphological characters consist of random mixtures of pure lines together with a number of heterozygous plants. The result is an equalization of differences between strains for all characters except rust resistance and other characters linked to it closely.

It is apparent that if all the emmer characters of the H-44-24 were carried by one chromosome and that if this chromosome had come over intact to the rust resistant lines, this material might be no better for comparative tests than different wheat varieties since there might still be incidental correlations between rust resistance and chemical compounds. The fact that the two types of rust resistance and several morphological characters have been shown to be inherited independently (1, 3) is evidence that the emmer characters did not come over in one chromosome and, consequently, the probability that a certain chemical compound or constitution characteristic of the emmer species is fortuitously linked with rust resistance in this material, is very much reduced.

When investigations reported in this paper were undertaken it was expected that time and staff would be available for extended analytical studies of these wheats. In these circumstances it appeared wise to prepare for more difficult examinations by first subjecting the wheats to a comparatively simple series of analyses designed to determine the main groups of plant compounds: fats, sugars, nitrogen compounds, hemicellulose, cellulose, lignin, etc. Although it seemed scarcely probable that differences related to rust reaction would be found between the wheats in these constituents, the work was undertaken in

the belief that it constituted the most logical approach to the problem under investigation.

Owing to the pressure of other work, these investigations now have to be dropped for some years at least. Meanwhile the results so far obtained are published in the hope that they may be of use to other investigators.

Materials

Production

A classification of the twelve lines of wheat described above, together with the letters by means of which each class will be identified throughout the rest of this paper, is presented in Table I. The seed for each class was made up by mixing equal weights of the seed of each of the three lines composing the class. The crop was grown on the experimental fields of the Department of Field Crops, University of Alberta, Edmonton, during 1933. The wheats were seeded in a block consisting of rod-row plots side by side. The block was divided into five sub-blocks, each containing four plots of three rows. Each sub-block contained one plot of each class of wheat, the classes being distributed at random within sub-blocks. All plots were seeded on May 2, 1933, at a rate of two bushels per acre. Three rows of Marquis wheat were seeded around the entire block.

TABLE I

CLASSIFICATION OF HYBRID LINES OF WHEAT SELECTED FROM A MARQUIS × H-44-24 CROSS

Winnipeg, 1929 Greenhouse No.	Rust reaction		Identification letters
	Seedling	Mature-plant	
113 125 135	Resistant	Resistant	RR
91 347 702	Resistant	Susceptible	RS
111 121 149	Susceptible	Resistant	SR
127 263 637	Susceptible	Susceptible	SS

Collection of seedling leaves

The first collection was made on May 27 when the plants were at the three-leaf stage. The leaves were cut off just above ground level, precautions being taken to see that no soil was included with the samples. A sample of 100 gm. green weight, was cut from each plot.

Collection of mature leaves

Mature leaves were collected on July 8 when the plants were well headed out. Samples were taken from plants which had not previously been cut. The leaves, with the exception of the terminal one (which does not exhibit the same degree of mature-plant resistance), were stripped off by hand. All dead and brown parts were discarded and a 100-gm. sample of green leaves was taken from each plot.

Methods

The analyses were carried out according to the system outlined by Waksman and Stevens (5), with the addition of a series of quantitative extractions with organic solvents. The analytical procedure is shown in the form of a flow sheet on page 5.

Dry matter

Since there were five plots of each class of wheat, determinations of air-dry matter were made in quintuplicate. Immediately after cutting, 100-gm. samples of the green leaves were placed in tared, loose, cheesecloth bags and dried first for 1.5 hr. at 110° C., then for three days at 65° C. After standing at room temperature for four days, the material reached an equilibrium with room conditions. It was then weighed and the percentage air-dry matter was calculated.

The material was then shipped to Ottawa where the five samples of each class of wheat were combined and ground in a Wiley mill to pass a 60-mesh sieve.

Oven-dry matter was determined in duplicate on the bulk samples by drying for 24 hr. *in vacuo* at 98° C.

Total ash

Determinations of total ash were carried out in duplicate by burning 1.5-gm. samples to constant weight in a muffle furnace at 600° C.

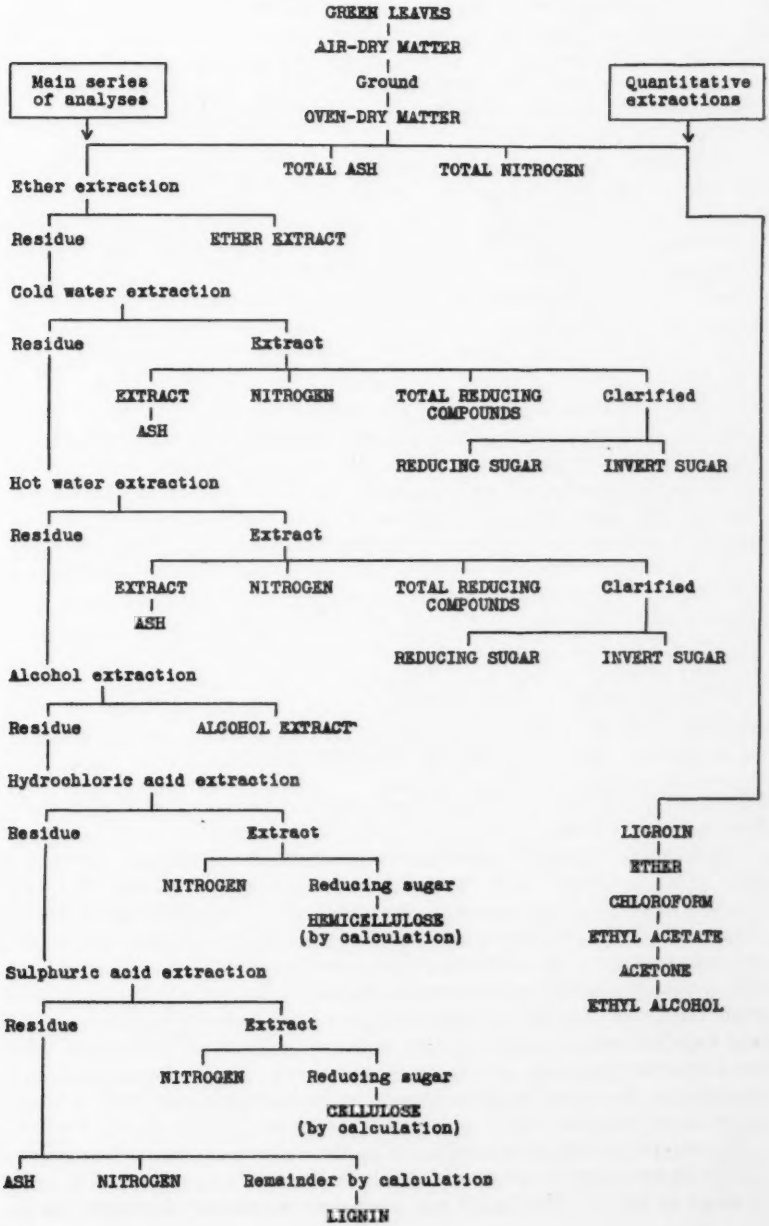
Total nitrogen

Determinations of total nitrogen were carried out on duplicate 2-gm. samples by the reduced iron modification of the Kjeldahl method (4).

Main series of analyses

The cold-water, hot-water, alcohol, and acid extractions outlined by Waksman and Stevens (5) are empirical and require the most painstaking technique if duplicate analyses are to check closely. Experiments showed that determinations made on different days were not directly comparable, owing, doubtless, to small and uncontrollable differences in conditions. In order to abolish this source of error, the eight samples, representing duplicates of each of the four wheat classes cut at the same stage of growth, were analyzed concurrently. In addition, the possibility of systematic errors, introduced by the order of precedence in handling, was minimized by randomizing the order in which the eight samples were dealt with throughout the various steps of the analytical procedure. Where heating was necessary, it was carried out in a mechanically stirred, electrically heated and controlled bath filled with either water or oil.

The cold- and hot-water extracts filtered slowly and fermented rapidly, thus increasing the difficulties of the determinations by setting time limits for the operation involved from the time the cold water was added to the sample until it was treated with alcohol. The speed of filtration was increased by extracting all samples in centrifuge bottles and by centrifuging and decanting


GREEN LEAVES
AIR-DRY MATTER
Main series of analyses
Ground
Quantitative extractions
OVEN-DRY MATTER
TOTAL ASH
TOTAL NITROGEN
Ether extraction
Residue
ETHER EXTRACT
Cold water extraction
Residue
Extract
EXTRACT
ASH
NITROGEN
TOTAL REDUCING COMPOUNDS
Clarified
REDUCING SUGAR
INVERT SUGAR
Hot water extraction
Residue
Extract
EXTRACT
ASH
NITROGEN
TOTAL REDUCING COMPOUNDS
Clarified
REDUCING SUGAR
INVERT SUGAR
Alcohol extraction
Residue
ALCOHOL EXTRACT
Hydrochloric acid extraction
Residue
Extract
NITROGEN
Reducing sugar
HEMICELLULOSE (by calculation)
Sulphuric acid extraction
Residue
Extract
NITROGEN
Reducing sugar
CELLULOSE (by calculation)
ASH
NITROGEN
Remainder by calculation
LIGNIN
LIGROIN
ETHER
CHLOROFORM
ETHYL ACETATE
ACETONE
ETHYL ALCOHOL

the extract on to the filter. Washing was accomplished by the same procedure, the remainder of the residue being transferred to the filter with the last washings. Fermentation was prevented for the required period by the use of chloroform water. The necessary technical accuracy and a standard programme for the execution of the various analyses were developed by means of preliminary experiments.

The initial weight of the samples taken for analysis was 5 gm. Ether extract was determined indirectly by the method described later. The cold-water- and hot-water-soluble matter were determined directly by evaporating aliquots of the extracts to dryness in Vitreosil dishes. Ash determinations were made on the residue in the same dish by the method previously described. Alcohol-soluble matter was determined by evaporating the whole of the extract to dryness in a glass beaker. The hydrochloric-acid- and sulphuric-acid-soluble matter were determined indirectly by weighing the sample before and after extraction. Nitrogen determinations were made on aliquots of the various extracts by the method previously described.

All sugar determinations were carried out on aliquots of the extracts by Hulme and Narain's (2) modification of the Hagedorn-Jensen-Hanes method. Clarification was effected with a minimum quantity of basic lead acetate solution (the quantity having been determined beforehand in a pilot experiment), and the solutions were de-leaded with sodium hydrogen phosphate. Total reducing compounds were determined by the same method without clarification. Inversion was carried out for 24 hr. at room temperature with hydrochloric acid (5 ml., sp. gr. 1.184, in 50 ml. of solution) and neutralization was effected by adding from a pipette 25 ml. of sodium hydroxide solution of the exact strength required to neutralize the acid solution.

The determinations of total reducing compounds in the cold- and hot-water extracts, and of the nitrogen in the acid extracts, were the only additions to the system of analyses outlined by Waksman and Stevens (5). In all other respects their directions were followed.

Quantitative extractions

The Soxhlet apparatus used for the quantitative extractions consisted of eight extractors, with interchangeable ground glass joints, arranged in a compact circle in a mechanically stirred, electrically heated and controlled water bath. The extractors were carefully selected for uniformity in size and rate of siphoning. Uniform boiling was promoted by placing a 1-cm. cube of pumice stone in the bottom of each flask. Extraction flasks, extraction units, condensers and pumice stone blocks were numbered and each set was kept together and was always placed in the same position in the bath. The stand for the extractors was made in such a way that all extractors were immersed to the same depth and could be lowered into the bath, or raised out of it, at the same time.

The determinations were carried out by the indirect method. The samples (initial weight 4 gm.) were dried, both before and after extraction, for 24 hr. *in vacuo* at 98° C. Extraction was continued for 48 hr. with each solvent.

After extraction with each solvent the replicate residues were mixed and re-sampled for the next extraction. This method was adopted to prevent the introduction of correlated errors.

The seedling and mature samples were analyzed in separate series, each of which consisted of either two or three sets of duplicate extractions of the four classes of wheat. Three sets of extractions were made with ligroin, ether and chloroform, and two with ethyl acetate, acetone and ethyl alcohol. The order in which the samples were weighed out for each set was randomized and the same order was maintained for all succeeding operations. Since the extractors were numbered and kept in order, the distribution of the samples in them was also randomized by this procedure.

Analytical Results

The analytical results are presented in Table II as the means of replicate determinations. The differences between means required for a 5% level of significance were calculated by statistical methods, and they are also reported in the table. Since these differences show the standard of accuracy attained in each determination, the publication of results of replicate determinations is unnecessary. In those cases in which the determination was not sufficiently precise to differentiate between any of the varieties the differences are enclosed in brackets.

With the exception of the quantitative extractions, all determinations were carried out in duplicate and concurrently on four classes of wheat collected at the same stage of growth. Four pairs of duplicate analyses were thus available in each case for statistical treatment. The standard deviation of the mean of duplicate determinations was calculated from these data and hence the necessary difference between means required for a 5% level of significance.

Quantitative extractions were carried out concurrently on duplicate samples of each of the four classes of wheat, collected at the same stage of growth. The extractions were then repeated either once or twice more. The data from either two or three sets of four duplicate analyses were thus available for statistical treatment. The data were subjected to an analysis of variance, the variance being divided into portions due to: (i) differences in the general level of results obtained in different sets of analyses, (ii) average differences, over all sets, between wheat classes, (iii) variations in differences between wheat classes from set to set, and (iv) differences between duplicate determinations within sets. The variance due to differences between sets of analyses as a whole was thus eliminated from the comparison of the wheat classes. The Z test was then applied to determine whether the variance due to wheat classes was significantly greater than those due to the interaction and the variance within sets. When the results of the Z test were positive the necessary difference between means, required for a 5% level of significance, was calculated in the usual manner.

TABLE II

ANALYSES OF HYBRID LINES OF WHEAT, DIFFERING IN THEIR RUST REACTIONS, AT TWO STAGES OF GROWTH

Determination	Wheat class, seedling				Necessary difference*	Wheat class, mature				Necessary difference*
	RR	RS	SR	SS		RR	RS	SR	SS	
Dry matter										
Air-dry matter as % of green wt.	18.40	18.77	18.48	18.53	(0.64)	24.81	24.41	23.94	23.61	0.96
Oven-dry matter as % of A.D. matter	92.24	92.40	92.28	91.49	0.08	94.17	93.84	93.79	93.87	0.12
Oven-dry matter as % of green wt.	19.95	20.31	20.03	20.25	—	26.35	25.80	25.52	25.15	—
Main series of analyses (Constituents as % of oven-dry matter)										
Total ash	14.13	13.58	14.12	13.65	0.09	8.18	8.22	8.32	8.27	0.05
Total nitrogen	6.19	6.15	6.13	6.23	0.03	2.25	2.35	2.30	2.24	0.02
Ether extract	5.09	5.24	5.23	5.40	0.09	2.28	2.21	2.26	2.13	0.03
Cold water extract	37.05	36.40	37.43	36.61	0.17	22.32	21.96	21.89	21.47	0.27
Ash	10.32	10.23	10.04	10.38	(0.76)	6.05	6.12	6.16	6.31	0.08
Nitrogen	1.51	1.40	1.48	1.48	0.03	0.90	0.93	0.94	0.91	0.03
Total reducing compounds	3.94	3.76	4.20	4.04	0.08	5.67	5.60	5.22	5.26	0.07
Reducing sugars	1.16	0.96	1.32	1.21	0.07	4.02	3.97	3.69	3.70	0.13
Invert sugar	6.63	7.07	7.01	6.83	0.15	3.31	2.75	3.09	2.91	0.11
Hot water extract	3.11	3.04	3.21	3.16	(0.33)	2.40	2.50	2.49	2.38	0.09
Ash	0.98	0.96	1.07	1.01	(0.12)	0.60	0.65	0.63	0.64	0.02
Nitrogen	0.16	0.15	0.15	0.16	(0.02)	0.07	0.08	0.07	0.07	0.01
Total reducing compounds	0.55	0.52	0.57	0.56	0.05	0.62	0.65	0.61	0.62	0.01
Reducing sugars	0.17	0.13	0.16†	0.17	—	0.37	0.37	0.36	0.36	(0.04)
Invert sugar	0.07	0.07	0.08†	0.08	—	0.08	0.07	0.09	0.08	(0.03)
Alcohol extract	3.37	3.57	3.36	3.41	0.11	2.31	2.25	2.39	2.21	0.05
Hydrochloric acid extract	34.29	34.49	33.66	34.13	0.25	33.28	33.28	33.04	33.37	(0.76)
Nitrogen	3.75	3.74	3.69	3.77	0.05	0.89	0.93	0.88	0.88	0.02
Hemicellulose	10.95	11.00	10.55	10.81	0.07	22.07	22.14	22.19	22.48	0.23
Sulphuric acid extract	12.21	12.19	12.07	12.36	(0.52)	28.78	28.51	29.41	29.47	0.62
Nitrogen	0.33	0.31	0.34	0.33	0.01	0.12	0.12	0.12	0.11	(0.02)
Cellulose	8.83	8.85	8.74	8.93	0.19	24.32	24.11	24.91	25.10	0.47
Residue	5.12	4.94	5.30	4.89	(0.49)	8.32	8.82	8.29	8.47	0.28
Ash	1.39	1.20	1.44	1.22	0.07	0.60	0.58	0.55	0.58	(0.09)
Nitrogen	0.31	0.30	0.31	0.31	(0.03)	0.20	0.22	0.20	0.20	0.01
Lignin (by difference)	1.83	1.87	1.97	1.78	(0.32)	6.48	6.40	6.52	6.64	0.19
Quantitative extractions (Constituents as % of oven-dry matter)										
Ligroin	3.49	3.51	3.63	3.66	0.04	1.66	1.64	1.61	1.55	0.04
Ether	1.62	1.70	1.55	1.64	0.04	0.61	0.59	0.64	0.63	0.03
Chloroform	2.05	2.22	2.02	2.11	0.09	0.96	0.75	0.82	0.83	0.11
Ethyl acetate	1.00	0.93	0.93	0.97		0.50	0.59	0.49	0.51	
Acetone	4.27	4.49	4.28	4.13		4.00	3.83	4.28	4.05	
Ethyl alcohol	13.83	13.46	13.73	12.67		10.23	9.86	9.57	9.67	

* *Necessary difference between classes for a 5% level of significance.*

( ) *No significant differences.*

† *Single determination only.*

Discussion

The series of analyses to which the wheats were subjected accounts for about 95% of the constituents of the dry matter of the leaves, provided that the last three nitrogen fractions are calculated as protein. The procedure separates the constituents into a number of main fractions and examines some of these in more detail. The determinations are empirical and the names given to the various fractions must be interpreted with due respect to the method of determination.

In the main series of analyses it became increasingly difficult to obtain duplicates that checked, owing to the cumulative effect of small errors. These effects were more noticeable in the seedling series in which the amount of material left for the later determinations was small. In the series of quantitative extractions the analyses also became progressively less accurate. This was not the result of cumulative errors, since these were avoided by resampling between extractions. As far as could be determined the errors are associated with the higher boiling points of the solvents used in the last half of the series. High boiling solvents emphasize differences in size, length and shape of the vapor tubes of the Soxhlet extractors with the result that differences in the rates of siphoning of the extractors are increased. With ethyl acetate, acetone and alcohol, the errors introduced by variations in siphoning rate were greater than the differences between the various wheats.

The four classes of wheat proved to be very similar in composition at each of the two stages of growth. Nevertheless the determinations were sufficiently accurate to show that differences occurred between the wheats in a large number of the fractions. As might be expected, the differences between the wheats at the seedling and mature stage were very considerable.

In examining the data for possible relations between chemical constitution and rust reaction, the nature of seedling reaction and mature-plant reaction must be borne clearly in mind. The former is really misnamed: it is a characteristic of the plant throughout its whole life. Mature-plant reaction, on the other hand, is well named. It represents a type of resistance which develops as the plant matures and which is superimposed upon the plant's original seedling reaction.

The combinations of wheat classes used in these studies and the fact that they were examined at two stages of growth, make it possible to examine the data with a view to determining whether quantitative differences in any of the fractions are related to either seedling reaction or mature-plant reaction. If a difference in the amount of any fraction is responsible for a difference in seedling reaction, then at both stages of growth the classes RR and RS probably should contain either more or less of that fraction than the classes SR and SS. On the other hand, if a difference in the amount of any fraction is responsible for a difference in mature-plant reaction, then there should be no differences between the classes at the seedling stage, and at the mature stage the classes RR and SR should contain either more or less than the classes RS and SS.

Careful examination of the data has failed to reveal any relation between any fraction and either type of rust reaction. This was to be expected in view of the fact that the fractions determined represent major groups of plant constituents rather than individual compounds. Nevertheless, the investigation, though its results are negative, forms the first step in a logical attack upon the rust problem by the forces of analytical chemistry, and paves the way for more detailed, difficult, and time-consuming investigations of the possible relations of individual constituents of the wheat leaf to rust reaction.

Acknowledgment

The writer is indebted to Dr. C. H. Goulden and Dr. K. W. Neatby (at that time) of the Dominion Rust Research Laboratory, Winnipeg, for supplying the hybrid lines of wheat; to Dr. O. S. Aamodt (at that time) Professor of Field Crops, University of Alberta, Edmonton, for supervising the growing of the wheats and the collection of samples, and to Mr. R. C. Rose, Research Assistant, National Research Laboratories, Ottawa, for carrying out some of the analyses.

References

1. GOULDEN, C. H., NEATBY, K. W. and WELSH, J. N. The inheritance of resistance to *Puccinia graminis tritici* in a cross between two varieties of *Triticum vulgare*. Phytopathology, 18 : 631-658. 1928.
2. HULME, A. C. and NARAIN, R. The ferricyanide method for the determination of reducing sugars. A modification of the Hagedorn-Jensen-Hanes technique. Biochem. J., 25 : 1051-1061. 1931.
3. NEATBY, K. W. Factor relations in wheat for resistance to groups of physiologic forms of *Puccinia graminis tritici*. Sci. Agr., 12 : 130-154. 1931.
4. PUCHER, G. W., LEAVENWORTH, C. S. and VICKERY, H. B. Determination of total nitrogen of plant extracts in presence of nitrates. Ind. Eng. Chem. (Anal. Ed.), 2 : 191-193 1930.
5. WAKSMAN, S. A. and STEVENS, K. R. A system of proximate chemical analysis of plant materials. Ind. Eng. Chem. (Anal. Ed.) 2 : 167-173. 1930.

A MICROSCOPICAL STUDY OF INFECTION OF THE ROOTS OF STRAWBERRY AND TOBACCO SEEDLINGS BY MICRO-ORGANISMS OF THE SOIL[1]

By A. A. Hildebrand[2] and L. W. Koch[3]

Abstract

Similarity of organisms encountered in studies of black root of strawberry and of tobacco, respectively, carried out contemporaneously but independently, suggested the co-operative investigation, the results of which are embodied in this paper. Strawberry and tobacco seedlings growing (i) in seed-bed muck heavily infested with *Thielaviopsis basicola* (Berk.) Ferraris, and other organisms known to be pathogenic on tobacco, (ii) in soil from a commercial plantation where strawberry root rot had occurred in severe and typical form, and (iii) in greenhouse compost soil, were examined microscopically daily, commencing a few hours after germination and continuing throughout a period of four weeks. Organisms observed definitely within root tissues of both hosts included the "phycomycetous mycorrhizal" fungus, *T. basicola* (observed in plants grown in muck only), *Rhizoctonia* (*Solani* and endophytic orchid types), forms of *Pythium*, *Asterocystis* (*Olpidiaster*), certain unidentified fungi, a minute filamentous alga and nematodes. Organisms observed on the surface of roots included representatives of the genera *Cylindrocarpon* (*Ramularia*), *Fusarium*, *Helminthosporium*, *Sphaeropsis*, and *Cephalothecium*. The sequence of appearance, percentage occurrence, and parasitic capabilities of certain of the organisms varied in roots grown in the different soils. Because of early infection by, and ultimate almost universal occurrence of, the phycomycetous mycorrhizal fungus this organism received especial attention. Evidence based on certain morphological differences suggests the occurrence of strains of this organism. Of interest, too, is an alga invading living root tissue.

From observations not limited alone to the examination of diseased roots of strawberry and tobacco, the authors are led to conclude, (1) that a root rot as it occurs in nature is extremely complex even in cases where a primary causal agent is recognized, and (2) that fungi representative of comparatively few groups or genera are "common factors" in root-rot complexes of different host plants.

The technique described offers distinct advantages in that it permits a study of the sequence and severity of infection by the organisms involved in a root-rot complex; it reveals the occurrence of obligate parasites the presence of which would never be detected by the soil-plating, the Cholodny, or the tissue-isolation methods; and it is readily adaptable to the study of other root-rot complexes.

Introductory

In the course of investigations on black root of strawberry (8) and of tobacco (11), respectively, carried out simultaneously but independently during the past two years at the St. Catharines laboratory, microscopical examinations of affected roots, supplemented by large numbers of isolations, have shown that representatives of the same genera or groups of fungi, as well as nematodes, are commonly associated with both diseases as they occur typically in nature on their respective hosts. This seemed of interest in view of the fact that in black root rot of tobacco, an annual plant, a single pathogen,

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namely, *Thielaviopsis basicola*, is regarded as the primary causal agent of the disease, whereas in black root rot of strawberry, a perennial plant, recent investigations and observations by Strong and Strong (15), Berkely and Lauder-Thomson (1), Truscott (16), Hildebrand (8) and Hastings (7) have shown that different primary parasitic organisms may attack the roots. In the light of our present knowledge, however, it is impossible to select a specific organism as the causal agent of the disease.

Of further interest is the fact that while both diseases may cause serious damage to older roots, their attack on roots in the primary condition is especially severe. A diseased condition and paucity of finer absorbing laterals are outstanding characteristics of black root rot of strawberries and it is well known that black root rot of tobacco may cause serious loss of seedlings. The similarity of the organisms encountered in these characteristically different root rots suggested to the present authors the advisability of a co-operative investigation on certain phases of the problem at least. The further fact that the attack of the two diseases on roots in the primary condition is especially severe, suggested the study of the roots of seedlings as the logical starting point. In the present paper are embodied, therefore, the results of an intensive microscopical study of the complete root systems of a large number of seedlings of tobacco and strawberry, employing a clearing and staining technique which renders possible a study of infection by, and recognition of, micro-organisms residual in the soils of plantations and seedbeds in which the respective diseases had occurred in severe and typical form.

Material and Methods

Two different soil types were used in the present study. The first, a muck, such as is more usually used in tobacco seedbeds, was chosen because it had already produced several crops of plants affected with black root rot and because, in addition to *T. basicola*, it was known to be heavily infested with the "phycomycetous mycorrhizal" fungus (8, 10, 11, 12, 16) and with representatives of other genera of fungi known to be pathogenic on tobacco (11). The second, an ordinary clay loam, for convenience hereinafter referred to as "strawberry-root-rot soil", was obtained from a commercial plantation where strawberry root rot had occurred for several seasons. It has already been reported in a previous publication (8) that when strawberry runners were trained into this soil, they developed into "degenerate" plants exhibiting typical symptoms of black root rot. Tobacco seed, variety Judy's Pride, and strawberry seed, variety Nich Omer, were sown in the greenhouse in 14-inch clay saucers containing, respectively, sterilized and non-sterilized soil of the two types. Both kinds of seeds were also planted in saucers of sterilized sand and in addition strawberry seeds were planted in greenhouse compost soil. To insure uniform stands, a weighed quantity of each kind of seed was sown in the respective saucers.

Commencing a few hours after the radicles had started to emerge from the testa and continuing for a period of four weeks, at the end of which time the

roots had developed laterals of the third and fourth order, seedlings of both strawberry and tobacco were removed daily from the soil and examined microscopically, a few unstained, but the majority cleared and stained using lacto-phenol with acid fuchsin added, according to the technique more recently employed by Truscott (16), Hildebrand (8) and Koch (11). The results which follow are based on the critical study of the complete root systems of over 350 seedlings of tobacco and 400 of strawberry.

Strawberry and Tobacco Seedlings Grown in Muck

Organisms Observed within Root Tissues

Strawberry and tobacco seeds planted in muck showed first signs of germination on the seventh and fifth days, respectively, following planting. Within 24 hours after germination, during which time radicles had developed to lengths ranging from 2–4 mm., specimens were first obtained for microscopical examination. The organisms observed within the tissues of these newly formed radicles and of the later formed roots included the following:

Thielaviopsis basicola

Four of ten radicles of strawberry, which could not possibly have been in contact with the soil for a period exceeding 18–24 hours showed infection by *T. basicola* (Plate I, Fig. 6). Reference to Table II will show that infection of tobacco seedlings by *T. basicola* was first observed on the fourth day after germination. Examination of Table I will show that of a total of 60 strawberry seedlings examined during the first 11 days 15% showed infection by *T. basicola* while 20% of the 79 tobacco seedlings examined during the same period showed infection by this organism. During the longer period, *i.e.*, from the 11th to the 30th day, 2.5% of the 40 strawberry seedlings and 75% of the 120 tobacco seedlings examined, showed infection by *T. basicola*. Of interest in this connection is the 55% increase in infection on tobacco, the naturally susceptible host, as contrasted with the 13% decrease in infection on strawberry, a host which would appear to be susceptible to infection by this organism only during early stages of development of the seedling.

In regard to the parasitism of *T. basicola* on the two hosts it was noted in the case of strawberry that although there was definite intracellular penetration there was no apparent necrosis of invaded cells, and infections did not progress beyond an incipient stage. In tobacco, on the other hand, the fungus was an aggressive parasite, producing the well known symptoms of black root rot.

Rhizoctonia

Infection by two distinct types of *Rhizoctonia*, namely, the *Solani* and orchid types (8, 11, 16) was observed on both strawberry and tobacco. During the first 11 days 17.5% of the tobacco seedlings examined showed infection by *Rhizoctonia* of the *Solani* type. During the subsequent 11- to 30-day period, infection by representatives of this type had decreased to 13.5%. Similarly in the case of strawberry during the two corresponding periods

TABLE I

PERCENTAGE OCCURRENCE OF MICRO-ORGANISMS IN AND ON ROOTS OF SEEDLINGS OF TOBACCO AND STRAWBERRY, COMPUTED FROM RESULTS OF DAILY MICROSCOPICAL EXAMINATION DURING 30-DAY PERIOD AFTER GERMINATION

	Tobacco	Strawberry	Tobacco	Strawberry	Tobacco	Strawberry	Tobacco	Strawberry	Strawberry
	Seed-bed muck				Strawberry-root-rot soil				Compost soil
Number of roots examined	79	60	120	40	50	100	45	70	12
Age of roots in days	1–11	1–11	11–30	11–30	1–11	1–11	11–22	11–22	1–21
Organisms within root tissues									
Phycomycetous mycorrhizal fungus	15.0	31.6	92.5	75.0	10.0	14.0	55.0	74.3	20.0
T. basicola	20.0	15.0	75.0	2.5	0.0	0.0	0.0	0.0	0.0
Rhizoctonia, Solani type	17.5	11.6	13.5	2.5	2.0	1.0	3.0	0.0	5.0
Rhizoctonia, orchid type	0.0	6.6	6.2	2.5	10.0	13.0	22.0	20.7	5.8
Pythium	16.2	0.0	8.2	2.5	12.0	16.0	9.3	12.8	3.3
Asterocystis	0.0	0.0	2.0	0.0	0.0	0.0	2.0	2.8	0.0
Alga	Frequent	Frequent	Frequent	Frequent	Frequent	Frequent	Frequent	Frequent	0.0
Unidentified	3.7	8.3	6.0	2.5	14.0	16.0	12.0	4.3	21.6
Nematodes	0.0	0.0	5.1	0.0	8.0	27.0	35.5	33.3	5.0
Organisms on surface of roots									
Cylindrocarpon	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0
Fusarium	1.0	1.6	3.0	2.5	3.0	0.0	3.0	1.4	0.0
Helminthosporium	0.0	0.0	0.0	0.0	5.0	2.0	7.0	1.4	0.0
Sphaeropsis	1.0	0.0	1.0	0.0	0.0	0.0	2.0	0.0	0.0
Cephalothecium	1.0	0.0	1.5	0.0	1.0	0.0	1.0	0.0	1.0
Unidentified	3.0	3.3	2.0	0.0	11.0	11.0	3.0	2.8	5.0
Roots free from infection	11.2	38.3	6.0	22.5	18.0	30.0	6.0	8.5	44.1

there was a decrease in infection from 11.6% to 2.5%. During the first 11 days tobacco was free from infection by the orchid type of *Rhizoctonia*, whereas the older seedlings examined showed 6.2% infection. This is in contrast with the recorded observations on strawberry. Younger seedlings of the latter host showed 6.6% infection, which decreased to 2.5% in the older seedlings.

Regarding the parasitism of *Rhizoctonia* on the two hosts it was noted in connection with the *Solani* type that penetration was more usually effected at or near the ground level and resulted in considerable damping-off in tobacco but scarcely any in strawberry. In both hosts, however, a sufficient number of clear-cut cases of tip infections resulting in necrosis and disorganiza-

TABLE II

SEQUENCE OF INFECTION OF ROOTS OF TOBACCO AND STRAWBERRY SEEDLINGS BY MICRO-ORGANISMS RESIDUAL IN THREE DIFFERENT TYPES OF SOIL

Days after germination	Muck		Strawberry root-rot soil		Greenhouse compost
	Tobacco	Strawberry	Tobacco	Strawberry	Strawberry
1		*T. basicola* *R. Solani*			
2				*R. Solani* Nematodes	Orchid Rhiz.
3			Orchid Rhiz.	Orchid Rhiz.	*R. Solani* *Pythium* Nematodes
4	*Pythium* *T. basicola*			*Pythium*	
5	*R. Solani*	Phyco. Mycor. Orchid Rhiz.		Phyco. Mycor. Orchid Rhiz.	
6	Phvco. Mycor. Orchid Rhiz.	*Pythium*			
7			Alga		Phyco. Mycor. Orchid Rhiz.
8			Nematodes		
10	Alga		*R. Solani*		
11			*Pythium*	Alga	
12		Alga	Phyco. Mycor. Orchid Rhiz.		
14	Nematodes				
15	Orchid Rhiz.				
25	*Asterocystis*		*Asterocystis*		
28				*Asterocystis*	

tion of tissue (Plate I, Fig. 1) was observed to indicate that representatives of the *Solani* type are capable of causing definite root rot as distinct from damping-off.

In both hosts, where infection by the endophytic orchid type of *Rhizoctonia* was observed, it was noted that even in heavily infected roots where many of the cells were completely filled with the mycelial complex of the fungus (Plate I, Figs. 4 and 5), there was relatively little discoloration or disorganization of cells or tissue invaded by the fungus. The lack of virulence displayed by the orchid type of *Rhizoctonia* was in marked contrast to that exhibited by members of the *Solani* group.

Pythium

Only 2.5% of 40 strawberry seedlings examined showed infection by *Pythium*, whereas 16.2% of the 79 younger and 8.2% of the 120 older tobacco seedlings showed infection by representatives of this genus. While in strawberry *Pythium* was relatively unimportant, nevertheless, in tobacco the observations indicated that members of the genus are important pathogens as regards both damping-off and root rot.

The "phycomycetous mycorrhizal" fungus

During the first 11 days 15% of tobacco and 31% of strawberry seedlings showed infection by the "phycomycetous mycorrhizal" fungus. By the end of 30 days, however, 92% of the tobacco and 75% of the strawberry seedlings showed heavy infection. Later, in this soil, it proved impossible to find root systems of either strawberry or tobacco free from invasion by this fungus.

Asterocystis (Olpidiaster)

A few of the older tobacco seedlings examined revealed the presence of *Asterocystis* but this obligate parasite was not observed in strawberry seedlings less than 30 days old.

Alga

Both in tobacco and in strawberry there was frequently observed in individual cortical cells, at or near the ground level, an organism which in stained material somewhat resembled a nematode both in size and general appearance, except that it was multiseptate. Examination of fresh material showed, however, that it was a chlorophyll-bearing organism, a member of the filamentous type of blue-green algae. As yet no effort has been made to identify this alga definitely with a view to determining its taxonomic position. Examination of a large number of roots invaded by this alga indicates that penetration is effected by mechanical means. Following direct penetration through the cell wall, the organism coils within the cell (Plate I, Figs 2 and 3). In no case has necrosis been correlated with the presence of the organism when it occurs alone. That it is not restricted to the soils used in the present experiments but is probably widely distributed in nature, is suggested by the fact that Koch has noted its presence in tobacco seedlings obtained from commercial seedbeds. An evaluation of the significance of the presence of

this organism either as a possible primary parasite, or as an agent providing an infection court for other organisms, has not yet been made. Meanwhile it must be regarded as a possible factor in the root-rot complex.

Nematodes

As reference to Table I will show, nematodes were not observed in the roots of strawberry and tobacco and only in about 5% of the older seedlings of the latter host.

Unidentified fungi

Of a total of 100 younger and older seedlings of strawberry examined, an average of 5.4% were found to be infected by fungi which could not be identified. The same was true for the 199 tobacco seedlings examined, the occurrence of unidentified fungi being slightly lower, namely an average of 4.8%. Since previous investigators (1, 15, 16) have shown certain imperfect fungi to be pathogenic on strawberry roots it is possible, particularly in the case of strawberry, that important pathogens may be included in the unidentified fungi, but since their mycelium within the tissues lacks the distinguishing characteristics necessary for even a generic diagnosis, it is impossible to evaluate the significance of their occurrence in a study of this kind.

Organisms Observed on the Surface of Roots

In addition to the organisms observed within the root tissues, certain fungi including representatives of the genera *Cylindrocarpon (Ramularia)*, *Fusarium*, *Helminthosporium*, *Sphaeropsis*, and *Cephalothecium* were also noted on the surface of roots. These fungi were for the most part identified by their conidia. The latter were frequently observed germinating on the surface of the roots but in no case was penetration by the germ tubes definitely noted.

Strawberry and Tobacco Seedlings Grown in "Strawberry-root-rot" Soil

Strawberry and tobacco seeds planted in the "strawberry-root-rot" soil showed the first signs of germination on the ninth and sixth days, respectively, after planting. As in the case of seeds planted in muck, examination of the seedlings was begun within 24 hours subsequent to germination. While the organisms observed within the roots of the seedlings of both hosts growing in strawberry-root-rot soil were more or less identical with those found in the roots of seedlings grown in muck—with the outstanding exception of *T. basicola* —nevertheless the percentage of occurrence of these organisms and the sequence of infection by them (Tables I and II), showed considerable variation in the two types of soil. The organisms which were definitely identified are recorded below in the same order as in the preceding section.

Rhizoctonia

While both the *Solani* and orchid types of *Rhizoctonia* were observed in both hosts, infection by the first-mentioned type occurred much less frequently. Not more than 3% of the tobacco seedlings, nor more than 1% of the strawberry seedlings showed infection by this fungus, though considerable surface

mycelium was observed. These percentages are in marked contrast to those calculated for infection in the muck soil. These observations would suggest that *R. Solani* as one of the components in the root-rot complex may vary markedly with soil type. Percentage infection by the orchid type of *Rhizoctonia* varied from 10–13 in the younger seedlings of tobacco and strawberry respectively, to 22 and 20 in older seedlings of the two hosts. Examination of Table I will show that these percentages are much higher than those recorded for seedlings grown in muck.

The observations recorded in the preceding section regarding the parasitism of the two types of *Rhizoctonia* on seedlings grown in muck confirm these made in connection with the seedlings grown in the strawberry-root-rot soil. It will be noted, however, that the relative occurrence of the two types of this organism varies with soil type.

Pythium

Whereas only 2.5% of 40 strawberry seedlings grown in muck showed infection by *Pythium*, 16% of 100 younger and 12.8% of 70 older strawberry seedlings grown in the root-rot soil showed infection by representatives of this genus. In the case of tobacco, however, there was a slight decrease from 24.4% of the 119 seedlings grown in muck to 21.3% of the 95 grown in the root-rot soil.

The examination of seedlings grown in root-rot soil indicates that *Pythium* is an important primary pathogen on both hosts. In many cases where root extremities were rotting off, *Pythium* alone could be found in the disintegrating tissues of the affected roots. As in the case of *Rhizoctonia*, while *Pythium* is undoubtedly the cause of damping-off, more especially in the case of tobacco, nevertheless, sufficient tip infections were observed to show that this fungus is responsible for definite root rot.

The "phycomycetous mycorrhizal" fungus

During the first 11 days 10% of the tobacco and 14% of the strawberry seedlings showed infection by the "phycomycetous mycorrhizal" fungus. These percentages by the end of 30 days had increased to 55 and 74.3. Later, as in the case of seedlings grown in muck, it was impossible to find root systems of either host free from invasion by this fungus.

Asterocystis (Olpidiaster)

Approximately the same percentage of *Asterocystis* was observed in the older roots of tobacco seedlings grown in the strawberry-root-rot soil as in those grown in muck. In the case of strawberry, whereas this fungus was not observed at all in roots of seedlings grown in muck, it was noted in 2.8% of older seedlings grown in the root-rot soil.

Alga

In the seedlings of both hosts grown in the strawberry-root-rot soil the alga to which reference has already been made in the preceding section was frequently observed.

Nematodes

One of the most striking differences in the occurrence of micro-organisms in the two types of soil was that noted in regard to nematodes. Whereas in muck these organisms were entirely lacking in the younger seedlings of both hosts and were observed in only 5% of the tobacco seedlings up to 30 days old, on the other hand, in the root-rot soil they were present in 8% of the tobacco and 27% of the strawberry seedlings up to 11 days old, these percentages increasing to 35.5 and 33.3 in older seedlings. Hildebrand (8) and Koch (11) have already suggested the importance of nematodes in the root-rot complex. In the present study they were observed alone and in association with various fungi. In many cases where they occurred alone necrosis of invaded cells indicated primary parasitism on the part of these organisms, while in certain cases, discoloration and at least partial disintegration of cells contiguous to those invaded suggested some such "action in advance" as, according to Goodey (6), was observed by Quanjer in the case of *Anguillulina dipsaci*, a parasite of potatoes and various other plants. Even though definite proof is lacking as to the primary parasitic capabilities of these organisms, in any case they must provide by mechanical means or otherwise, ideal infection courts for facultative parasites which otherwise could not establish themselves within the host. Where nematodes and fungi were found together, even in incipient infections it was impossible to determine which of the organisms had gained entry first.

The observations in general in regard to nematodes suggest that these organisms are an important factor in the root-rot complex, more particularly, perhaps, as it obtains in the case of strawberry.

Unidentified fungi

As reference to Table I will show, the percentage of roots in which unidentified fungi were observed, was higher for seedlings grown in strawberry-root-rot soil than for those grown in seed-bed muck. Thus, of a total of 170 strawberry seedlings examined, an average of 10.1% showed infection by unidentified fungi as compared with an average of 5.4% in strawberry seedlings grown in seed-bed muck. Of 95 tobacco seedlings grown in the strawberry-root-rot soil, an average of 13.0% showed infection by unidentified fungi, whereas an average of only 4.8% of those grown in seed-bed muck were infected by fungi which could not be identified.

Organisms observed on the surface of root tissues

In regard to organisms which were observed on the surface of root systems, what has been said for seedlings grown in muck also holds true for seedlings grown in the root-rot soil.

Strawberry Seedlings Grown in Greenhouse Compost Soil

Of one hundred and twenty 1- to 21-day-old strawberry seedlings grown in greenhouse compost soil, 44% were found to be healthy and the rest remained relatively freer from infection than those grown in the muck and the root-rot soil. The "phycomycetous mycorrhizal" fungus was observed in 20% of

the seedlings examined. Primary infection by the fungus was first noted on the seventh day, as compared with the fifth day in the other two types of soil. Infection by the two types of *Rhizoctonia* was also appreciably less in the compost soil, the *Solani* and orchid types being observed in only 5 and 5.8% of the seedlings, respectively. The alga which had been noted so frequently in seedlings grown in muck and in the root-rot soil was not observed at all in those grown in the compost soil. Nematodes were noted in 5% of the seedlings and they differed in type from those observed in the seedlings from the other two soils. Very few organisms were noted on the surface of the roots of the seedlings grown in the compost soil.

The "Phycomycetous Mycorrhizal" Fungus in Tobacco and Strawberry

Of all the organisms that could be definitely identified within the tissues of the roots of tobacco and strawberry, the phycomycetous mycorrhizal fungus was the most conspicuous in appearance and the most consistent in occurrence. The widespread distribution of this organism in strawberry, together with suggestions as to its possible significance as a factor in the root-rot complex has been pointed out by O'Brien and McNaughton (12), Truscott (16), and Hildebrand (8). More recently Koch (11) has shown that the same or a closely similar organism is present in the roots of tobacco, in the thalli of certain liverworts, and in the stems and leaves of certain mosses. Opinions vary as to the significance of the presence of this fungus in regard to pathological conditions in the strawberry. O'Brien and McNaughton (12), and Truscott (16) conclude that this endophyte is not only a definite parasite but is an extremely important pathogen of the strawberry. In regard to tobacco the evidence adduced by Koch (11) indicates that the fungus possesses parasitic capability on this host.

In view of the fact that the endophyte in question seemed to indicate not only different pathogenic capabilities on tobacco and strawberry, but also exhibited certain morphologic differences on the two hosts, it was decided to study the organism more critically with a view to obtaining additional information, whatever its nature, concerning this obligate parasite which, never having been grown in pure culture, cannot be studied directly.

Infection and development stages

In regard to infection by, and developmental stages of, the endophyte, reference to Table III will show that initial infection was first noted in tobacco grown in muck, six days, and in strawberry five days, after germination. In root-rot soil initial infection of tobacco was noted 13 days, and in strawberry five days, after germination. In greenhouse compost soil initial infection of the latter host was first noted seven days after germination. In tobacco, arbuscules appeared two to three days after initial infection while in strawberry they appeared three to four days after initial infection in all three soil types. In this connection it is of interest to note

that whereas Reed and Fremont (14), in their study of endotrophic mycorrhiza in the roots of citrus, observed arbuscules in certain cases only, particularly "where cells appear to be in complete physiological equilibrium (trees regularly receiving a complete fertilizer)", in the present studies arbuscules were especially abundant in both hosts regardless either of host vigor or of soil type. In tobacco, both in muck and in root-rot soil, vesicles appeared 15 days after germination. In strawberry, on the other hand, the appearance of vesicles seemed correlated with soil type, since in seedlings grown in muck, in root-rot soil and in compost they appeared in 9, 14 and 19 days respectively after germination of seeds. The "sporangiole" stage was not observed within the four-week period of intensive examination of seedlings.

TABLE III

INITIAL INFECTION BY, AND DEVELOPMENTAL STAGES OF, THE "PHYCOMYCETOUS MYCORRHIZAL" FUNGUS IN THE ROOTS OF TOBACCO AND STRAWBERRY SEEDLINGS, GROWN IN THREE DIFFERENT SOILS

First appearance of	In tobacco		In strawberry		
	In muck, days*	In strawberry-root-rot soil, days	In muck, days	In strawberry-root-rot soil, days	In compost, days
Infection	6*	13	5	5	7
Arbuscules	8	16	8	9	10
Vesicles	15	15	9	14	19
Necrosis of host tissue	17	18	—	—	—

* *After germination.*

In regard to the vesicles, the present investigations shed no further light as to the true nature of these structures, that is, whether they are resting bodies, storage organs or sporangia. Peyronel (13) in 1923, and Van der Pijl (17) in 1934, recorded the presence of spores in the vesicles of the endophyte. The critical examination of hundreds of vesicles in both stained and unstained material of different ages has revealed within these bodies the presence of numerous small oil globules which in untreated material suggest the delimitation of the contents into spore-like bodies, but which, as Truscott (16) has already pointed out, when treated in lacto-phenol, coalesce into one large globule.

Ontogenetic Relations

Early in the investigation it was casually noted in the case of tobacco that infection by the endophyte occurred anywhere from the root tip to the root-stem transition, whereas, in strawberry, infection either in the immediate region of the root tip or the root-stem transition was of rare occurrence. This seemed of interest in that there was suggested in the case of strawberry a correlation between infection and ontogeny of the root.

A critical examination of 100 roots of each host up to 21 days old was made to compare the distribution of infection especially in relation to root tip and root-stem transition. The results obtained are shown graphically in Fig. 1. It will be observed from an examination of Fig. 1, A, that in tobacco not only

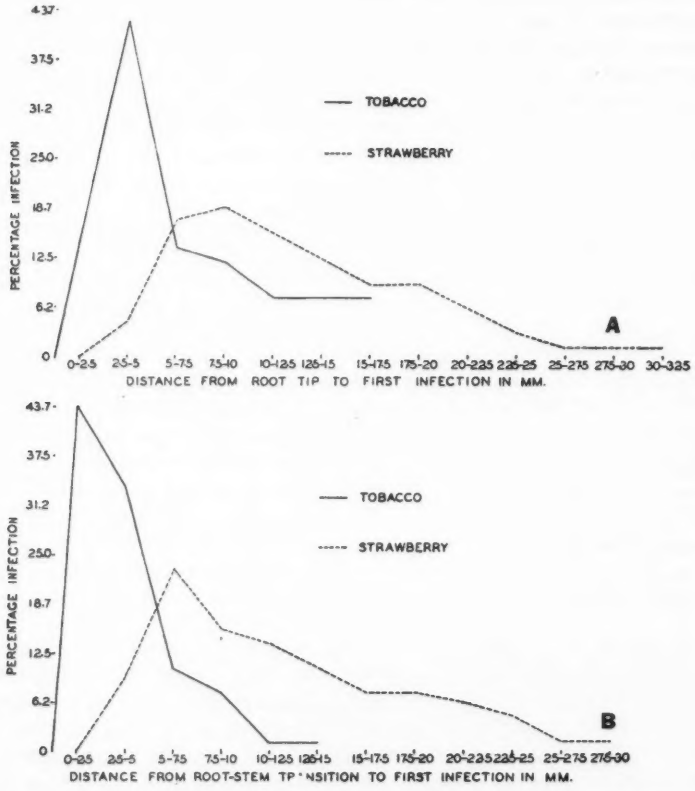


FIG. 1, A and B. *Curves showing percentage occurrence of most distal and most proximal infections in relation to root tip and root-stem transition.*

did tip infection occur but that the maximum of the most distal infections, 41%, occurred within the range of 2.5–5 mm. from the root tip and that only 7% of the most distal infections occurred within the range of 15.0–17.5 mm. from the root tip. On the other hand, in the case of strawberry, only a single case of tip infection was observed and the maximum of the most distal infections, 18.7%, occurred within the range of 7.5–10.0 mm. from the root tip, with a small percentage of the most distal infections occurring within the range of 30.0–32.5 mm. from the root tip.

With regard to the most proximal infections, examination of Fig. 1, B, will show that in tobacco the maximum of such infections, 43.7%, occurred within the range of 0–2.5 mm. from the root-stem transition and that only 1% of these infections occurred within the range of 12.5–15 mm. from the root-stem transition. On the other hand, in strawberry, the maximum of the most proximal infections, 23%, occurred within the range of 5.0–7.5 mm. from the root-stem transition, while only 1% occurred within the range of 27.5–30.0 mm. Between these two limits most proximal infection decreased in direct proportion to the increasing distance from the root-stem transition.

From the above results it would appear, then, that in strawberry in the regions of both the root tip and the root-stem transition, tissues of seedlings are resistant to infection by this fungus. In tobacco, such regions free from infection do not exist. The reason for the apparent immunity of certain regions of the roots in the case of strawberry is not known. It is possible, however, that some such correlation exists between ontogeny and infection, as Fitzpatrick (5) has demonstrated in the case of infection of peach leaves by *Taphrina deformans*.

It should be pointed out that in the region intermediate between the most distal and the most proximal infections heavy infection usually occurred in the roots of both hosts.

Morphological differences between the endophytes in the two hosts

Early in the present investigation it became apparent that certain morphological differences existed between the organism noted in tobacco and that observed in strawberry. In strawberry, when a strand of extramatrical mycelium comes in contact with the surface of the root an irregularly shaped appressorium-like enlargement of the mycelium very often develops (Plate II, Fig. 1). From the appressorium-like enlargement a wedge-like hypha develops, pushing its way between the epidermal cells (Plate II, Figs. 1 and 2). From this advancing and penetrating hypha, mycelium develops which ramifies in the outer cortical cells (Plate III, Fig. 4). Coiling of mycelium in these earlier invaded cells was not observed. On the other hand, in tobacco, when an extramatrical strand of mycelium comes in contact with the surface of the root, in many cases such a strand continues along the surface, at intervals producing infection hyphae which penetrate the epidermal cells and which, instead of ramifying in all directions, as in the case of strawberry, produce characteristic coils in the invaded cells (Plate III, Fig. 3). From these coils, some of which are also formed in the outer cortical cells as well as in the epidermal cells, hyphae ramify in the underlying cortical tissues.

In the deeper cortical tissues of the strawberry invaded by this fungus a characteristic paralleling of intercellular mycelium was noted, which was lacking in the corresponding tissues of tobacco. A further difference was noted in the degree of septation of the mycelium. In tobacco, septa were of relatively much more frequent occurrence than in strawberry (Plate III,

Figs. 3 and 4). Short papilla-like protuberances are frequently characteristic of the mycelium of the strawberry endophyte (Plate II, Fig. 3) but were not noted in the case of the tobacco organism.

The arbuscules of the organism in strawberry (Plate II, Fig. 4) and tobacco are essentially similar but marked differences were noted in the case of the vesicles. The latter in strawberry are essentially more or less oval (Plate III, Fig. 2) and, as Truscott (16) has already pointed out, "tend to be somewhat distorted in the intercellular spaces". In tobacco the vesicles are typically sphaeroidal (Plate III, Fig. 1; Plate II, Fig. 5) though in this host as well, irregularly-shaped vesicles are sometimes observed. Six hundred and seventy-seven vesicles in tobacco were examined critically as to their shape. Of this number 474 (70%) were sphaeroidal, 125 (18%) were more or less oval (in this respect resembling more closely those observed in strawberry), and 78 (11%) were irregular in shape. The same number of vesicles were examined in strawberry and more than 95% were more or less oval in shape, the remainder being irregular, conforming to the configurations of the intercellular spaces. Intracellular vesicles were of much more frequent occurrence in tobacco than in strawberry.

Regarding the parasitism of this fungus on strawberry and tobacco, it should be pointed out that while in the latter host considerable necrosis of tissue could be correlated with the presence of the endophyte, in strawberry, on the other hand, necrosis was seldom observed though depletion of starch in invaded cells was a common occurrence. In the cortical region of tobacco, necrosis of invaded cells was consistently observed in cases where intracellular vesicles were present (Plate II, Fig. 6).

In summarizing the data presented in the foregoing paragraphs it is believed that sufficient evidence has been presented to warrant the view that the fungus which has hitherto been referred to as the "phycomycetous mycorrhizal" fungus is not a specific entity but consists of strains which can be distinguished more particularly on a basis of morphological differences.

Discussion

As Eaton and King (4) in their study of the cotton root-rot fungus have recently pointed out, "progress in soil microbiology has been handicapped by the lack of methods which give a true picture of the numbers, activities and relationships of the organisms under field conditions". It is believed that the technique described in the present investigations promises not only to assist in overcoming the handicaps referred to above, but in permitting the direct observation of sequence and relative degree of infection, also contributes materially to our knowledge of two outstandingly important root-rot diseases. While the present studies were conducted in the greenhouse there is no reason why the methods employed could not be modified for studies of root rots of the same or other host plants as they actually occur in nature. The method undoubtedly has its limitations since, for example, the organisms which can be observed are for the most part restricted to those which are pathogenic,

and thus it is impossible to evaluate the relationship, antagonistic or otherwise, existing between these pathogenic organisms and the numerous other micro-organisms of the soil. Information regarding these relationships may be obtained, however, by supplementing the technique described in the present paper with the method devised by Cholodny (2) and used subsequently with some measure of success by Jensen (9), Eaton and King (4) and Demeter and Mossel (3).

Of interest in the present investigations is the fact that in the two characteristic types of root rot, that is, one in which a chief primary causal organism is recognized and the other in which are undoubtedly a number of pathogenic organisms, the sequence and relationships of which are not yet clearly understood, there is a surprising similarity in regard to the organisms encountered. It may be mentioned here that incidental to the present studies, the roots of a number of other host plants affected with root rot, including tomato, lettuce and tulip have been examined microscopically and the same complex picture involving infection by the same or very similar organisms was observed. The constant association of similar organisms in root rots of widely different hosts would indicate that there is a certain rather limited microfauna and microflora concerned with root rots generally. The sequence of infection by the various organisms associated in the complex and the evaluation of their relationship not only to one another but also to the other micro-organisms concerned in the microbiological equilibrium of the soil remains for further research.

In the body of the present paper considerable emphasis has been placed on the "phycomycetous mycorrhizal" fungus. It is believed, however, that such emphasis is warranted in view of the fact that this fungus is so universally present in the roots of the two host plants under investigation. An intensive study of this fungus as it occurs in these two hosts has revealed certain consistent morphological differences which point strongly to the occurrence of strains of the organism. There is the possibility, however, that such differences as have been observed may be due to the effect of the host on the fungus.

The technique described in the present paper seems of especial value for the reasons that (1) it is a quick, effective method readily adaptable to the study of root rots in general, (2) it permits a study of the sequence of infection by the organisms involved in a root-rot complex from the earliest stages of root development, and, (3) it reveals the occurrence within the root tissues of obligate parasites, the presence of which would never be detected by the soil plating, tissue isolation, or Cholodny methods.

References

1. Berkeley, G. H. and Lauder-Thomson, Isabel. Root rots of strawberry in Britain. The "black lesion" type of strawberry root rot. J. Pomology Hort. Sci. 12 : 222-246. 1934.
2. Cholodny, N. Ueber eine neue Methode zur Untersuchung der Bodenmikroflora. Arch. Mikrobiol. 1 : 620-652. 1930.
3. Demeter, K. J. and Mossel, H. Uber die Brauchbarkeit von Cholodnys "Aufwuchsplattenmethode" bei mikrobiologischen Boden-Untersuchungen. Centr. Bakt. Parasiten. 88 : 384-393. 1933.

4. EATON, E. D. and KING, C. H. A study of the cotton root rot fungus (*Phymatotrichum omnivorum*) in the soil by the Cholodny method. J. Agr. Research, 49 : 1109-1113. 1934.

5. FITZPATRICK, R. E. The life history and parasitism of *Taphrina deformans*. Sci. Agr. 14 : 305-326. 1934.

6. GOODEY, T. The pathology and aetiology of plant lesions caused by parasitic nematodes. Bull. Imperial Bureau of Agr. Parasitology, St. Albans, England. 1935.

7. HASTINGS, R. J. Miscellaneous note on some nematode diseases of plants in British Columbia. Plant Dis. Reporter, 19 : 26-27. 1935.

8. HILDEBRAND, A. A. Recent observations on strawberry root rot in the Niagara peninsula. Can. J. Research, 11 : 18-31. 1934.

9 JENSEN, H. L. Contributions to the microbiology of Australian soils, II. A comparison of the Rossi-Cholodny method and the plate method for studying the soil microflora. Proc. Linn. Soc. N.S.W. 59 : 200-211. 1934.

10. JONES, F. R. A mycorrhizal fungus in the roots of legumes and some other plants. J. Agr. Research, 29 : 459-470. 1923.

11. KOCH, L. W. Recent investigations on root rot of tobacco in Canada. Can. J. Research, C, 13 : 174-186. 1935.

12. O'BRIEN, D. G. and MCNAUGHTON, E. J. The endotrophic mycorrhiza of strawberries and its significance. West of Scotland Agr. Coll. Research Bull. 1 : 1-32. 1928.

13. PEYRONEL, B. Fructification de l'endophyte à arbuscules et a vésicules des mycorrhizes endotrophes. Bull. Soc. Mycol. France 39 : 119-126. 1923.

14. READ, H. S. and FREMONT, MME. TH. The arbuscules of endotrophic mycorrhiza. Compt. Rend. Soc. Biol. 116 : 201-202. 1934.

15. STRONG, FORREST C. and STRONG, MIRIAM C. Investigations on the black root of strawberries. Phytopathology, 21 : 1041-1060. 1931.

16. TRUSCOTT, J. H. L. Fungous root rots of strawberry. Can. J. Research, 11 : 1-17. 1934.

17. VAN DER PIJL, L. Die Mycorrhiza von Burmannia und Epirrhizanthes und die Fortpflanzung ihres Endophyten. Rec. Trav. Bot. Neerlandais 21 : 761-779. 1934. Abstract in Rev. Applied Mycol. 14 : 248. 1935.

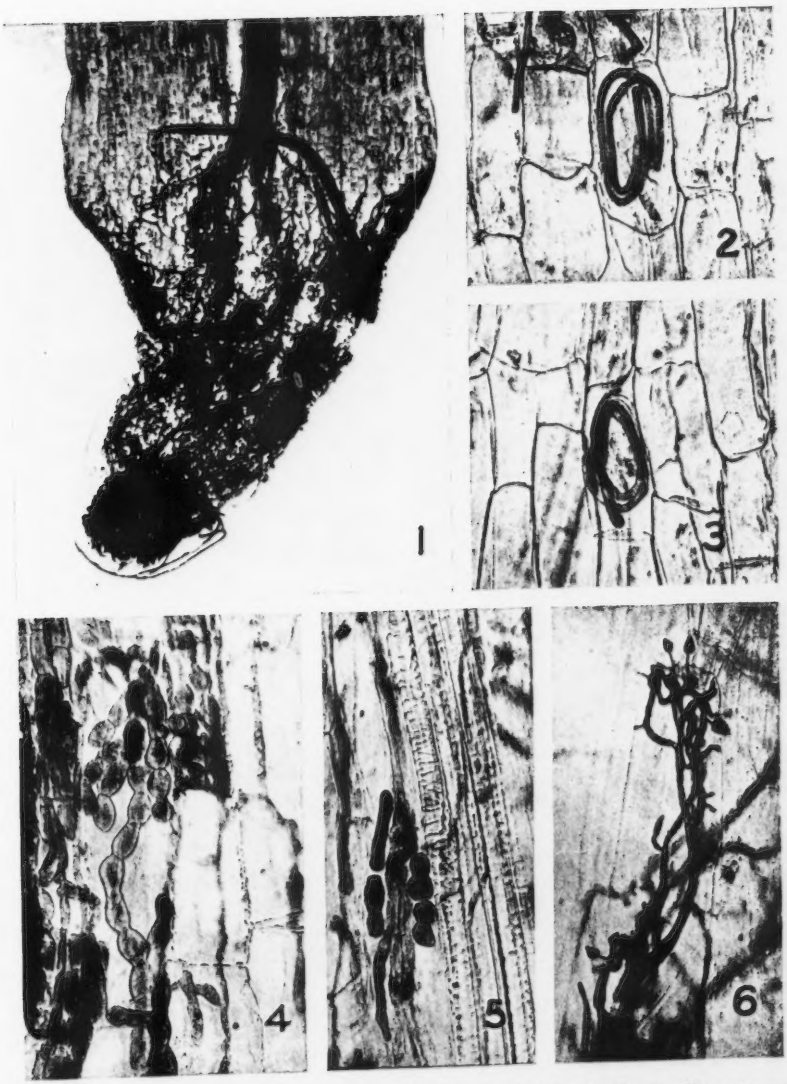
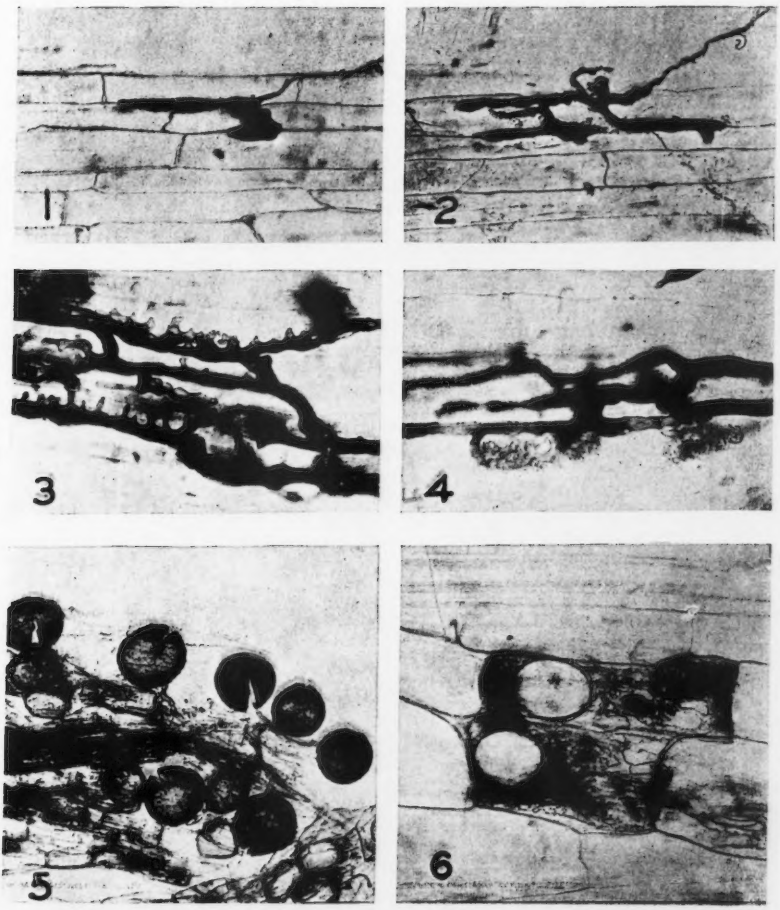
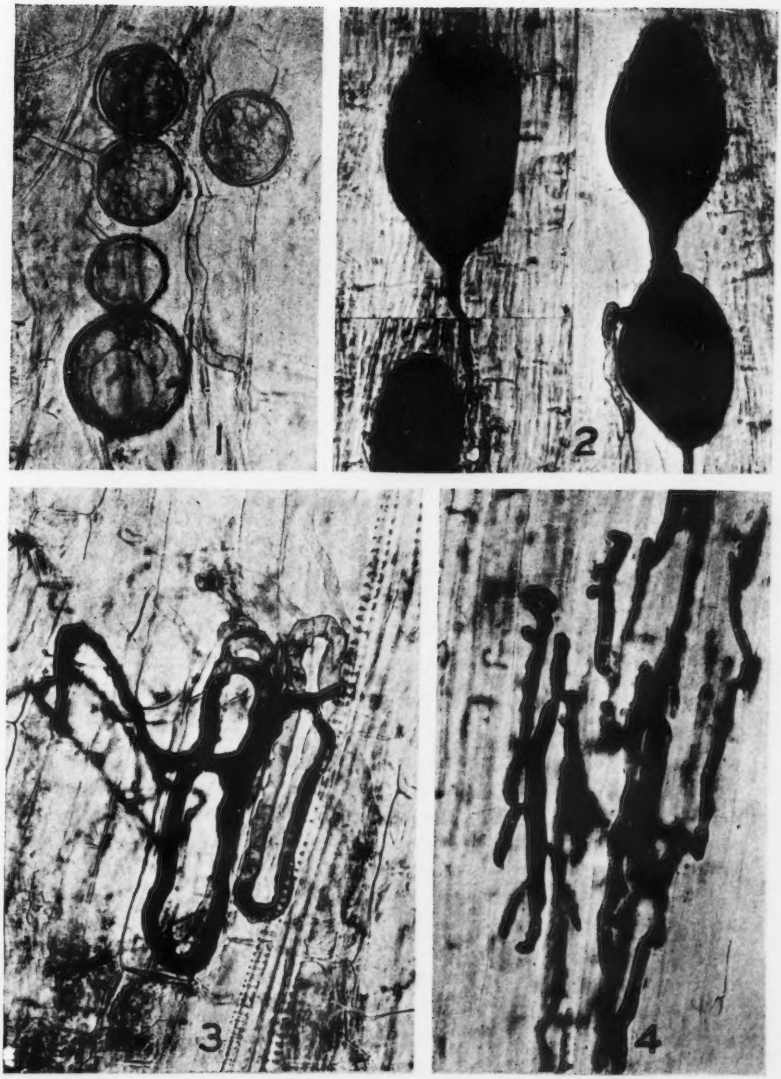


FIG. 1. *Tip of rootlet of strawberry seedling infected with Rhizoctonia Solani. Note presence also of a few nematodes*(× 70). FIGS. 2 AND 3. *Alga in outer cortical cells of rootlets of strawberry and tobacco, respectively* (× 360). FIGS. 4 AND 5. *Orchid type of Rhizoctonia infecting cortical cells of rootlets of strawberry and tobacco, respectively.* (×360). FIG. 6. *Thielaviopsis basicola infecting outer cortical cells of rootlet of strawberry seedling grown in naturally infested seed-bed muck* (× 360).



"Phycomycetous mycorrhizal" fungus in roots of strawberry (Figs 1–4) and of tobacco (Figs. 5 and 6). (All × 340). FIG. 1. *Early stage of infection showing strand of extramatrical mycelium and appressorium-like enlargement from which wedge-like penetrating hypha is pushing its way (towards the left) between epidermal cells.* FIG. 2. *Later stage of infection. Note wedge-like penetrating hypha at lower left.* FIG. 4. *Later stage of infection showing formation of first arbuscules.* FIG. 3. *Showing papilla-like protuberances commonly observed on mycelium of strawberry endophyte.* FIG. 5. *Tobacco rootlet showing late stage of infection. Note typically sphaeroidal vesicles.* FIG. 6. *Invaded cells of tobacco rootlet showing necrosis correlated with presence of intracellular vesicles.*



Photomicrographs showing outstanding morphological differences between the "phycomycetous mycorrhizal" endophyte as observed in the rootlets of tobacco (Figs. 1 and 3) and of strawberry (Figs. 2 and 4). FIG. 1. *Typical sphaeroidal vesicles of tobacco endophyte (× 340).* FIG. 2. *Oval-shaped vesicles typical of strawberry endophyte (× 360).* FIG. 3. *Early stage of infection by tobacco endophyte showing characteristic coiling of mycelium in outer cortical cells. Note also presence of septa (× 340).* FIG. 4. *Corresponding stage of infection by strawberry endophyte showing mycelium ramifying in outer cortical tissues without coiling. Note absence of septa (× 340).*

PEACH CANKER INVESTIGATIONS

II. INFECTION STUDIES[1]

By R. S. Willison[2]

Abstract

Two species of *Valsa* have been isolated more or less consistently from cankers of various ages, and from "die-back" twigs on the peach. In culture, one species, identified as *Valsa leucostoma* (Pers.) Fr., is hair-brown and has small dark pycnidia exuding cirri when mature. On the host, its stroma is compact in texture, contains no host cells and is delimited beneath by a black zone of carbonized fungal and host cells. Ascospores of *V. leucostoma* measure 10–17 by 2–4.5 μ. The other species, which has been assigned to *V. cincta* Fr., is whitish to olive buff in culture and has large light-colored pycnidia containing, though rarely exuding, spores. On the host, the stroma of *V. cincta* is comparatively loose in texture, contains host cells and is delimited from the cortex of the host by a thin, black zone, sometimes only marginal. Ascospores of *V. cincta* measure 14–28 by 4–7 μ. In both species, the pycnospores range from 5 to 10 μ in length and 1 to 2 μ in width. These organisms, along with *Sclerotinia fructicola* (Wint.) Rehm., were used in series of infection experiments at frequent intervals over a period of two years. Similar series of checks were also provided. Periodical observations and measurements furnished detailed case histories of all wounds concerned. *V. cincta* was found to be a virulent wound-parasite, able not only to infect freshly made wounds during the late autumn, winter and spring, but also to give rise to perennial cankers. Infection with this organism rarely occurred during June, July and August. *V. leucostoma* proved in these experiments to be almost, if not quite, incapable of initiating cankers on the peach. *S. fructicola* parasitized the tissues of branches and produced considerable necrosis during the first three weeks after inoculation during the growing season. Subsequently the lesions proceeded to heal. The degree of infection and the amount of resultant necrosis in wounds inoculated with *S. fructicola* during the dormant season were dependent upon the conditions of temperature and humidity then prevailing. *S. fructicola*, while capable of inducing lesions on the stem, cannot be regarded as the cause of typical peach canker. Some of the factors influencing infection by the three organisms mentioned above are briefly discussed.

In the first paper of this series (55), a number of factors contributing to the occurrence of canker on peach trees in the Niagara Peninsula were discussed and it was pointed out that the disease was due to the activities of a fungous wound-parasite. The present article deals with infection experiments concerning three fungi, two of which have been considered (23, 24, 28, 35, 44, 51) as the causal organisms of peach canker and of certain forms of "die-back". To avoid confusion, attention is directed at present to that phase of the disease manifested as cankers on larger branches, leaving for future consideration the phase affecting twigs, and its relation to the general subject of "die-back".

Much has been written in recent years concerning canker, gummosis, "die-back", "apoplexy" and kindred ailments of the stone fruit trees. However, the literature on the subject is to a large extent controversial, partly because of the tendency to seek a common cause for these troubles on the

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various hosts, and partly because these terms are sometimes employed to denote the same disease and sometimes entirely different ones. The following conclusions reached by certain investigators will serve to illustrate the existing confusion as well as the necessity both for further fundamental work and for the correlation and evaluation of existing information. Pfeiffer (40) attributes gummosis of peach, cherry, plum and apricot in Saxony to unfavorable cultural conditions rather than to bacterial invasion. Shilberszky (46) considers the dying-off of stone fruit trees to be due to the obstruction of conducting elements by tyloses and gummification associated with "abnormal enzymatic activity", to the physiological effects of severe frosts, especially those of late winter and spring, to unfavorable soil conditions, or to deep planting. In a later paper, (47) he extends the causes to include wounding, incompatible stocks and parasitic infection, especially by *Clasterosporium carpophilum* and *Sclerotinia cinerea*. At the same time, he claims to have refuted the view that *Cytospora* spp. and *Valsa* spp. are implicated in gummosis except in trees already weakened from other causes. Czarnecki (14), Van der Meer (52), Dufrénoy (16, 17), and Joëssel (29) suggest that infection by *Verticillium* spp. is an important factor in the dying-off of apricot and peach trees. However, Joëssel (29) and Joëssel and Bordas (30) consider that the disease is frequently associated with injuries and that *Verticillium* is only one of several causes. Faes and Staehelin (21) have not so far obtained evidence of the pathogenicity of species of *Clasterosporium*, *Monilia*, *Fusarium*, and *Verticillium* in the dying-off of apricots. On the other hand, Rives (41, 42) has demonstrated that "apoplexy" of apricot may be caused by bacterial organisms. While Curzi (13) submits evidence of wilting of peaches due to infection by *Phytophthora* spp., *Fusicoccum persicae* is cited as a possible cause of twig cankers of peach by Overholts (39) and *Nectria cinnabarina*, of "die-back" of apricot by Dowson (15).

The problem of gummosis, canker, "die-back", dying-off and/or "apoplexy" of stone fruits is evidently one of considerable complexity, presenting numerous phases, one or more of which have been investigated by each of the workers referred to above. At this point, it might be well to reiterate that the causal factors applicable to the disease complex on one host are not necessarily applicable to that on closely related hosts. This idea has already been broached by Miss Cayley (6) in connection with the behavior of *Cytospora* sp. and *Diaporthe perniciosa* on stone fruits, and later by Chabrolin (8) and Joëssel and Bordas (30) in reference to "apoplexy" of apricots. While peaches and apricots, on the one hand, and plums and cherries on the other, seem to have much in common in this regard, specific differences should not be overlooked. Regional variations in climate, soil and so forth should be considered, as they doubtless play their part in determining the factor or combination of factors operative in various districts. Thus, in order to be able to diagnose particular cases adequately and to recommend suitable control measures, it would seem necessary to take into account all factors which might be involved.

The almost constant association of *Cytospora* spp. and of *Valsa* spp. with canker and "die-back" in stone fruits has led investigators to consider that members of these genera are the primary agents. Aderhold (1) was one of the first to prove that *Cytospora* spp. could become established in dead areas on cherry and thence parasitize healthy tissues. A little later, Rolfs (44) succeeded in infecting, by cross inoculation, peach, plum, cherry, wild cherry and apricot with strains of *Cytospora* and *Valsa* isolated from each of these hosts. However, the times and methods of his inoculations, and even the organism used, are not always clear. But other workers have not been satisfied that "die-back" and canker are primarily due to these organisms. Miss Cayley (6) demonstrated that *Diaporthe perniciosa*, though only weakly parasitic, was more vigorous than *Cytospora* spp. on stone fruits, with the exception of peach. After failing to induce typical lesions on plum with *Cytospora* spp., Wormald (57, 63) showed that *Pseudomonas Mors-Prunorum* was the cause of canker, and frequently of death, in plum trees, although the lesions were soon overrun with *Cytospora* sp. Wilson (56) described a similar bacterial canker on plum, cherry, apricot and peach. On the other hand, McCubbin (35) was able to induce cankers in the peach with an organism designated as *Valsa leucostoma*. He introduced pieces of the organism from cultures under a V-shaped flap of bark, and then covered the wound with cloth dipped in grafting wax. He also found that, by artificially freezing the tissue prior to inoculation, infection was more easily achieved and the resulting damage greater. This experiment, however, cannot be taken as proving anything more than the ability of the *Valsa* sp. to establish itself in dead tissue. Togashi (51) accomplished similar results by inoculating branches of both peach and apricot with *Leucostoma Persoonii* (Nitsch.) Togas' i (syn. *Valsa leucostoma* (Pers.) Fr.) and with *Valsa japonica* Miyabe & Hemmi, by way of small cross slits cut with a scalpel hot enough to kill some of the adjacent tissue. He used spore suspensions as inoculum, covered the wounds with moist absorbent cotton and paraffin paper for five days, wetted the cotton daily, and afterwards painted the wound surface. Even with this method, he was unable to infect Terada plum and had only partial success with Golden Sugar plum. When a cold scalpel was used for making the incisions, infection did not occur even in peach (inoculated August 10, 1927). Unfortunately only one attempt was made with the cold scalpel, although the other method was employed in eight experiments between May 30 and November 14, 1927.

In a number of recent papers, considerable evidence has been presented to the effect that there is a marked seasonal variation in the ability of specific parasites to infect the woody parts of their host. In the case of wound-parasites, infection occurs most readily during the dormant season. Brooks and Moore (4) showed that *Stereum purpureum* can readily attack fresh wounds on plum at any time except June, July and August. Wormald (63) found *Pseudomonas Mors-Prunorum* infecting plum most effectively during the period from October to December but not at all from April to September.

Togashi (51) considered the critical period for infection of the peach with *Leucostoma Persoonii* and *Valsa japonica* to extend from the middle of August to the middle of November. Homma (26) reported similar results for *Camarosporium persicae* on peach and *Prunus mume*. Wilson (56) obtained more extensive bacterial cankers on plum and cherry by inoculating in late autumn and early spring, than he did in early autumn or late spring. Curzi (13) found that *Phytophthora* spp. gained entry to tissues during the dormant period, from October to spring. By way of contrast, Koch (31) demonstrated that the critical period for infection of plum twigs with *Dibotryon morbosum* is limited to May and June, the season of most vigorous growth. *D. morbosum*, however, exhibits a highly specialized type of parasitism which is quite distinct from that of the other organisms referred to.

The Fungi

In the course of the present investigations many isolations have been made from the internal tissues of cankers of various ages, and later, of monospores from fruiting bodies appearing on cankers and dead twigs. Predominant among the fungi cultured were two forms of *Cytospora*, easily distinguishable in culture, but separable only with difficulty on the host. In March 1931, with the discovery of perithecial stromata on the host, it was possible to identify these organisms as the imperfect stages of two species of *Valsa*. One agreed closely with descriptions, by Saccardo (45), Ellis and Everhart (18), and Togashi (50), of *V. leucostoma* (Pers.) Fr. The other has been tentatively designated as *V. cincta* Fr. as described by Saccardo (45) and Ellis and Everhart (18). Both species have been isolated frequently from cankers of several years' standing, though very rarely from the same canker. On the other hand, incipient cankers yielded *V. cincta* and *V. leucostoma* in the ratio of more than 20 : 1. Indeed, certain forms of incipient canker, notably lesions in the vicinity of buds, contained only *V. cincta*, except in a few cases where other organisms such as *Alternaria* spp. had become established in the dead tissue. *V. cincta* has also been isolated from incipient cankers on the apricot.

Valsa cincta and *V. leucostoma* are so similar in some respects, yet so dissimilar in others, that it seems desirable to point out the features by which each can be recognized. To facilitate comparison, descriptive data are presented in parallel in Table I. In this connection, it should be recognized that the object is not so much to present a complete description of these species of *Valsa* as to emphasize the salient differences between them. While the perithecial stromata of *V. cincta* and *V. leucostoma*, by virtue of differences in size of ascospores, are readily distinguishable on the host, the pycnidial stromata are not, and separation is impossible on the basis of measurements of pycnospores. However, if perithecial material is not available, as is often the case, the distinctive characteristics of the two organisms on artificial media provide a ready means either of recognition or of confirmation of identification by other means. Rolfs (44) identified the organisms with which he worked as *Valsa leucostoma* var. *rubescens*, and *V. leucostoma* var.

cincta, separated on the basis of slight cultural differences and of pycnospore sizes. His descriptions, indeed, support the idea that both were forms of *V. leucostoma*. However, his remark that "on peach and plum agar, white, hairy pustules occasionally formed, which were at first mistaken for perithecial stromata, but examination showed them to be pycnidial stromata" suggests that, at times, he did have *V. cincta*.

TABLE I

COMPARATIVE DESCRIPTIONS OF *Valsa cincta* FR. AND *V. leucostoma* (PERS.) FR., OCCURRING ON PEACH IN THE NIAGARA DISTRICT OF ONTARIO

	Valsa cincta Fr.	*Valsa leucostoma* (Pers.) Fr.
	On nutrient media	
Characteristics of cultures on potato-dextrose agar (5% dextrose) and on Leonian's* malt agar.	Surface of culture felty, white at first, later Tilleul Buff to Olive Buff (Ridgway), sometimes black in substrate. Very little aerial mycelium. Pycnidia large (1–3 mm. in diam.) white, felty, rarely if ever exuding cirri, though usually full of viable spores. (Plate I, Fig. 4.)	Surface of culture suede-like, at first white, later Hair-brown (Ridgway) or darker. Almost no aerial mycelium. Pycnidia small (1 mm. or less in diam.) rostrate, usually dark, exuding cirri when mature. (Plate I, Fig. 6.)
	Both organisms produce pycnidia more readily in cultures exposed to daylight than in those kept in the dark.	
	On the host	
Erumpent disc of stroma.	Muddy gray, usually circular, ostioles of perithecia, when present, usually peripheral, frequently surrounding the mouth of a pycnidium. (Plate I, Fig. 1.)	White at first, turning gray with age, frequently elliptical. Ostioles of perithecia, when present, central without regular order. (Plate I, Fig. 7.)
Stroma	Dark ectostromatic tissue not well developed. Comparatively loose in texture. Host cells prevalent in stroma *above* pycnidium and perithecia. Separated from subjacent cortical tissue of host by thin "black zone" of carbonized fungal and host cells, sometimes only marginal. (Plate I, Figs. 2 and 3.)	Considerable dark ectostromatic tissue beneath bark. Compact in texture. Host cells few or absent in stroma *above* pycnidium or perithecia. Separated from subjacent cortical cells of host by "black zone" of carbonized fungal and host cells, usually well developed. (Plate I, Figs. 5 and 8.)
Pycnidia	Multilocular with common ostiole. Definite wall, outer layers usually not carbonized. (Plate I, Fig. 3.)	Multilocular with common ostiole. Definite wall, outer layers sometimes carbonized on upper side of pycnidium, sometimes confluent with stroma. (Plate I, Fig. 5.)
Perithecia	Usually globose, sometimes compressed laterally. Circinate, frequently surrounding a pycnidium. Whole perithecial stroma usually on larger scale than in *V. leucostoma*. (Plate I, Fig. 2.)	Usually spheroidal, sometimes compressed longitudinally. Circinate, usually not in same stroma as pycnidium. (Plate I, Fig. 8.)
Pycnospores	(All spore measurements were made from fresh distilled water mounts from newly collected material.)	
	Allantoid 5.5–10 × 1–2 μ (mode 7 × 1.5 μ)	Allantoid 5.0–10 × 1–2 μ (mode 7 × 1.5 μ).
Ascospores	Allantoid 14–28 × 4–7 μ (occasionally longer). (Mode 20 × 5 μ).	Allantoid 10–17 × 2–4.5 μ (mode 13 × 3 μ).

* *Leonian's malt agar (32):— Primary potassium phosphate 2.4 gm.; magnesium sulphate 1.2 gm.; peptone 1.2 gm.; maltose 12.5 gm.; malt extract 12.5 gm.; agar 25.0 gm.; water 1000 cc.*

The reported pathogenicity of *Valsa leucostoma* under certain conditions (1, 6, 11, 22, 35, 44, 51, 53) and the frequency of the association of *Valsa cincta** with incipient cankers rendered it necessary to employ both species in inoculation studies, especially as preliminary trials had indicated a decided difference in their respective pathogenic capabilities. For several reasons, it was considered advisable also, to make parallel inoculations with *Sclerotinia fructicola* (Wint.) Rehm. (syn. *S. americana* (Worm.) Nort. & Ez.), the causal organism of brown rot of stone fruits in America. There are numerous references (2, 9, 10, 23, 24, 28, 33–36, 38, 43, 48, 49) to twig killing and bark injury due to this fungus, both on peach and on other stone fruits, while other members of the genus cause similar injury on pome and stone fruits in Europe and elsewhere (3, 12, 19, 20, 25, 27, 29, 37, 58–62). In the course of the present investigations many cases of twig blight and injury following both *Sclerotinia* blight of blossoms and brown rot of fruit have been observed. In 1913, failing to induce infection with *Valsa* spp., but succeeding with the brown rot organism, Jehle (28) came to the conclusion that *S. cinerea* (Bonn) Schroet. (as *S. fructicola* was then designated in America) was the cause of canker in peaches in New York State. Indeed the disease has often been referred to as "Brown rot canker". McClintock (33) observed the production of conidia of *S. fructicola* on stem lesions during the second season, while Manns and Adams (36) and Berkeley (2) reported the over-winter survival of blossom-blight cankers. On the other hand there has been considerable evidence, (5, 35) that *S. fructicola* is rarely, if ever, able to perpetuate cankers over a number of seasons. McClintock (34) in 1929 failed to discover any conidia on blossom-blight cankers in their second year. Roberts and Dunegan (43), after six years' experience, found that while the fungus remained alive over winter in a certain percentage of cankers each year, conidia were not produced (with one exception). They do not mention the enlargement of blossom-blight cankers during the dormant season following infection.

By means of repeated sub-culturing on Leonian's malt agar (32) a single strain of each of the organisms, *Valsa leucostoma*, *V. cincta* and *S. fructicola*, was used throughout the entire series of inoculations described below. The culture of *V. leucostoma* came from a single pycnospore but was indistinguishable from cultures of ascosporous origin. The culture of *V. cincta* originated from a single ascospore, while the strain of *S. fructicola* employed here was isolated from the wood of a branch beneath a brown rot mummy. Because of the differences in the time factor involved in the production of pycnidia, on one hand, and of hyphomycetous conidia on the other, the cultures of the *Valsa* spp. were from six to ten weeks old, while those of *S. fructicola* were approximately two weeks old when used for inoculation. To provide inoculum, spore suspensions of *V. leucostoma* and *S. fructicola* were easily obtained by shaking tube cultures covered with sterile distilled water, but in the case of *V. cincta*, the pycnidia had to be sliced with a sterile scalpel in order to liberate the pycnospores.

* *According to Schilberszky (47), Dr. A. Schellenberg considered Valsa cincta responsible for a die-back of peach. However, as all efforts to trace this reference have so far proved fruitless, it is impossible to give the citation here.*

Methods of Inoculation

Commencing August 24, 1931, successive series of inoculations were made, on trees of the Elberta variety, at fortnightly intervals until August 12, 1932. From then until October 6, 1933, the experiment was continued with the intervals ranging from one week, at critical times of the year, to one month. Thus the complete experiment covered slightly more than two calendar years and comprised 45 series. Each series consisted of four sets, one for each organism and one for check, distributed one set per tree. The technique was as far as possible uniform throughout, so that the variable factors were limited, in the main, to developmental phases of the host, and to meteorological conditions at the time of inoculation as well as to unavoidable differences in the trees themselves. The duration of the experiment through two complete growth cycles made it possible to check to a considerable extent the effects of the host phase at the time of inoculation. The comparatively large number of series tended both to provide a wide range of weather conditions and to minimize the effects of differences in host material. There remains, of course, the possibility of a decline in the virulence of the fungi as a result of repeated sub-culturing on artificial media. However, if any such loss of pathogenic ability occurred, it was so slight as to be imperceptible.

The procedure followed during inoculation was simple. For each set, five branches, usually of different ages and sizes, were surface sterilized for a distance of about four inches by brushing thoroughly with a tooth brush dipped in a 1 : 500 solution of mercuric chloride. These areas were then washed with a stream of freshly distilled water. A small piece of tissue, approximately one and a half by one-half inches, was then removed from each in such a manner that the long axis of the "blaze" was parallel to that of the branch. A cold scalpel, sterilized with mercuric chloride and wiped with a clean cloth, was used to make the incisions. Several drops of inoculum were then placed on the newly made wound by means of a medicine dropper. To approximate natural conditions as closely as possible, all wounds were left exposed to the weather. The obvious advantages of the absence of protective wrappings were considered to offset the possibly enhanced likelihood of chance infection and contamination. However, the non-inoculated wounds not only provided a check for this point but also served to indicate the behavior of uninfected wounds. During the first year (Series 1 to 25) each set also contained five pruning cuts, made without previous sterilization and inoculated in the same manner as the other wounds. In part of the second year (Series 32 to 45) a single slit, also about an inch and a half long but involving no removal of tissue, was substituted in each set for one of the "blazes" described above. All wounds in Series 31 were slits.

Observations

To preserve the identity of the wounds for purposes of observation, each one was labelled with an identifying tag and its location indicated on a diagrammatic sketch of the tree concerned. The original size of each wound

was recorded and its subsequent measurements ascertained from time to time. The latest observations and measurements were taken in October or November 1934 so that the history of the earliest series has been closely followed for three consecutive years and that of the latest for one year.

The data thus obtained are assembled in Figs. 1, 2 and 3. Fig. 1 indicates for each set of inoculations the percentage of wounds (i) increasing in size during the season in which inoculation took place, (ii) enlarging as active cankers during the dormant season of 1933–34 or at removal, (iii) enlarging

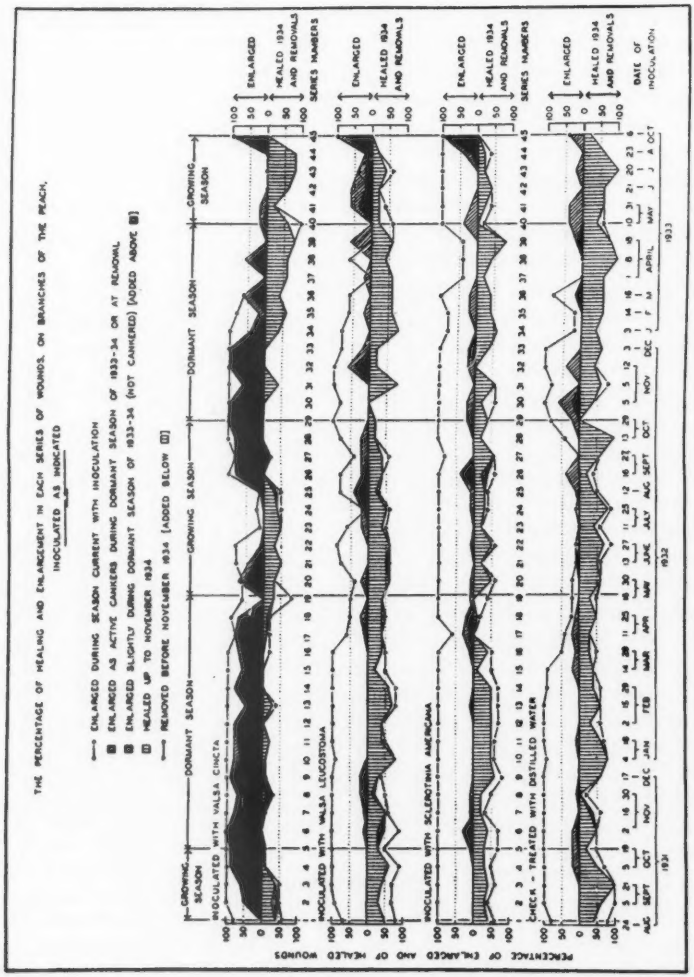
THE PERCENTAGE OF HEALING AND ENLARGEMENT IN EACH SERIES OF WOUNDS ON BRANCHES OF THE PEACH, INOCULATED AS INDICATED
ENLARGED DURING SEASON CURRENT WITH INOCULATION
ENLARGED AS ACTIVE CANKERS DURING DORMANT SEASON OF 1933-34 OR AT REMOVAL
ENLARGED SLIGHTLY DURING DORMANT SEASON OF 1933-34 (NOT CANKERED) [ADDED ABOVE ■]
HEALED UP TO NOVEMBER 1934
REMOVED BEFORE NOVEMBER 1934 [ADDED BELOW □]
GROWING SEASON
DORMANT SEASON
INOCULATED WITH VALSA CINCTA
INOCULATED WITH VALSA LEUCOSTOMA
INOCULATED WITH SCLEROTINIA AMERICANA
CHECK - TREATED WITH DISTILLED WATER
PERCENTAGE OF ENLARGED AND OF HEALED WOUNDS
ENLARGED
HEALED 1934 AND REMOVALS
SERIES NUMBERS
DATE OF INOCULATION
1931
1932
1933

FIG. 1. *The percentages of wounds healed, enlarged and cankered in various series inoculated at different times of the year with Valsa cincta, V. leucostoma and Sclerotinia fructicola respectively, and checks.*

slightly, but not cankered during 1933–34, (iv) completely healed in November 1934, and (v) removed prior to November 1934. To avoid confusion, enlargements, except in the case of the earlier removals, appear above the base line while healed wounds and removals appear below. The measurements of wounds of the first type described in the preceding section are summarized in Figs. 2 and 3. Data relevant to the measurements of the pruning wounds have not been considered, in the first place, because it was thought advisable to deal only with comparable wounds throughout, and, in the second place,

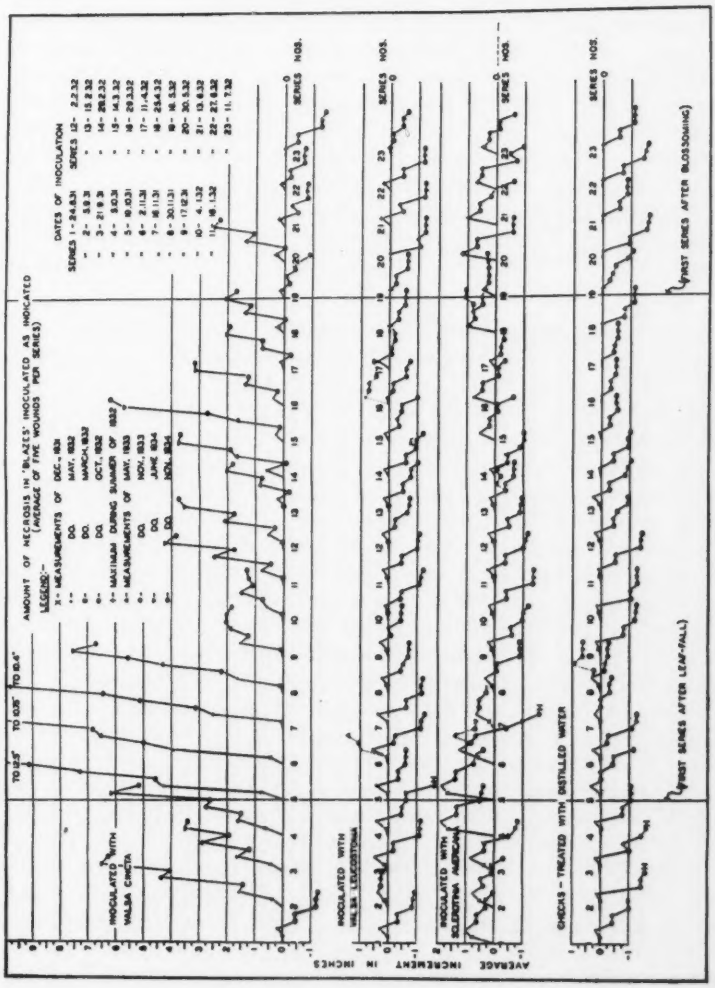
AMOUNT OF NECROSIS IN "BLAZES" INOCULATED AS INDICATED
(AVERAGE OF FIVE WOUNDS PER SERIES)
LEGEND:-
DATES OF INOCULATION
SERIES NOS.
INOCULATED WITH VALSA CINCTA
INOCULATED WITH VALSA LEUCOSTOMA
INOCULATED WITH LEUCOSTOMA PERSOONII
CHECKS - TREATED WITH DISTILLED WATER
AVERAGE INCREMENT IN INCHES
(FIRST SERIES AFTER LEAF-FALL)
(FIRST SERIES AFTER BLOSSOMING)

FIG. 2. *Records of progressive changes in the average size of wounds in each of Series 1 to 23, inoculated at different times of the year with V. cincta, V. leucostoma and S. fructicola respectively, and checks.*

because the behavior of the pruning wounds agreed in essence with that represented here. As necrosis advanced at a much greater rate at the ends of the wound parallel to the long axis of the branch, changes in the length of wounds were chosen as criteria for determining the progress of infection. Thus, each point on the various curves indicates the average length of a given set of wounds at the stated time, minus the average original length of the same wounds. As a consequence, the base lines represent average original lengths; positive ordinates, enlargements; and negative ordinates, decrease in size.

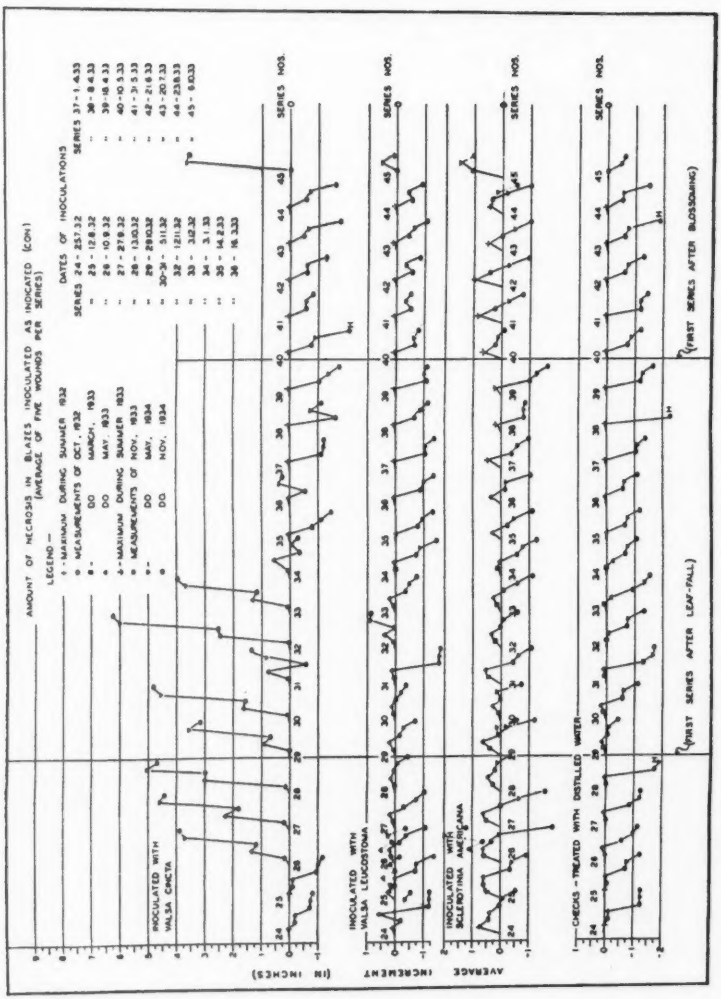
AMOUNT OF NECROSIS IN BLAZES INOCULATED AS INDICATED (CON.)
(AVERAGE OF FIVE WOUNDS PER SERIES)
LEGEND —
1 - MAXIMUM DURING SUMMER 1932
o MEASUREMENTS OF OCT., 1932
" DO MARCH, 1933
" DO MAY, 1933
Δ - MAXIMUM DURING SUMMER 1933
o MEASUREMENTS OF NOV., 1933
" DO MAY, 1934
" DO NOV., 1934
DATES OF INOCULATIONS
SERIES 24 - 25.7.32
" 25 - 12.8.32
" 26 - 10.9.32
" 27 - 27.9.32
" 28 - 13.10.32
" 29 - 29.10.32
" 30-31 - 5.11.32
" 32 - 12.11.32
" 33 - 3.12.32
" 34 - 3.1.33
" 35 - 14.2.33
" 36 - 16.3.33
SERIES 37 - 1.4.33
" 38 - 8.4.33
" 39 - 18.4.33
" 40 - 10.5.33
" 41 - 31.5.33
" 42 - 21.6.33
" 43 - 20.7.33
" 44 - 23.8.33
" 45 - 8.0.33
INOCULATED WITH VALSA CINCTA
INOCULATED WITH VALSA LEUCOSTOMA
INOCULATED WITH SCLERONIMA AMERICANA
CHECKS - TREATED WITH DISTILLED WATER
AVERAGE INCREMENT (IN INCHES)
SERIES NOS.
(FIRST SERIES AFTER LEAF-FALL)
(FIRST SERIES AFTER BLOSSOMING)

FIG. 3. *Records of progressive changes in the average size of wounds in each of Series 24 to 45, inoculated at different times of the year with V. cincta, V. leucostoma and S. fructicola respectively, and checks.*

For any one set of inoculations, the temporal sequence is shown, in a somewhat arbitrary fashion, by placing each successive measurement at a regular interval to the right of the preceding one. Consequently, the behavior of each set of inoculations is portrayed as a curve the upward tendency of which denotes necrosis and the downward tendency, healing. In those cases where, to avoid overlapping, the final reading or so has been omitted, that reading is the same as the last one which appears.

With these points in mind, even a cursory glance at these figures reveals fundamental differences in the reactions not only of the two *Valsa* species but also of *Sclerotinia fructicola*. Both the high percentage of active cankers developed in wounds inoculated at certain seasons of the year and their conspicuous and usually progressive enlargement are ample evidence of both the virulence of *V. cincta* under the conditions of the experiment and its ability to cause perennial cankers. On the other hand, *V. leucostoma* may be considered either as non-pathogenic or at best as an extremely weak wound-parasite. On the basis of active cankers produced, *S. fructicola* appears to rank with *V. leucostoma*, but the history of the wounds inoculated with the former organism reveals its strong though unsustained parasitic tendencies.

Discussion

V. cincta

It is of interest in connection with the present investigation, as close scrutiny of Figs. 1, 2 and 3 will show, that infection with *V. cincta* occurs most readily during the dormant season and for about six weeks before leaf fall, but only occasionally, if at all, during the height of the growing season, (compare Plate II, Figs. 1 and 2, Plate III, Figs. 6 and 7 with Plate III, Fig. 3). For a period in the late fall and early winter, determined largely by temperature conditions, *V. cincta* is able, shortly after inoculation, to cause visible damage, which progresses more or less steadily during the winter (Series 2–7, 26–28, Figs. 2 and 3). In the fall of 1931 this period lasted until November 25 during which time the mean daily temperature was usually well above 45° F. and sometimes as high as 60° F. The weather conditions prevailing for the corresponding season in 1932 were not so favorable for the fungus, as the mean daily temperature except for a few brief intervals, did not rise above 45° F. after October 29 (Series 29, Fig. 3). In the winter, when the mean temperature remained lower than 40° F., necrosis did not appear until the advent of warm weather in the spring (Series 8–13, 29–34, Figs. 2 and 3). This phenomenon is probably due to the inactivity of the host rather than to that of the fungus since there was a more or less steady decline in the initial amount of necrosis in successive sets of inoculations (Series 8–17, 32–35, Figs. 2 and 3). Moreover, Togashi (51) has demonstrated that internal temperatures in peach branches in winter frequently are considerably higher than the air temperature and often much above 32° F. Thus it may be argued that, in spite of generally unfavorable conditions and the consequent slowing down of fungal growth, opportunities are afforded during the winter months for spores to germinate and for the fungus to establish itself in the

host. It is logical, therefore, that the great extension of lesions during March and April is the result partly of the rapid growth of the fungus under the stimulus of more favorable temperatures and partly of the greater enzymatic activity of the host, inducing the discoloration which differentiates the infected from the healthy tissues. It is also reasonable to suppose that the amount of necrosis occurring at this time depends upon the foothold gained by the fungus during the winter, which in turn depends upon both the length of time and the conditions since inoculation. The discrepancy apparent in the behavior of *V. cincta* in Series 29 and 30 (Fig. 3) may in part be accounted for by the fact that, by reason of certain cultural practices (55), the trees involved were somewhat slower growing and hardier than those used in the subsequent series. In Series 31, (Fig. 3), as already noted, all wounds were slits which reacted somewhat differently than did the regular type of wound.

The failure of *V. cincta* to infect peach branches during a large part of the growing season may be explained by the interaction of three main factors, temperature, condition of the host, and the parasitic capabilities of the fungus. There is no doubt that this organism is unable to infect actively growing host tissue, but there is reason to believe that it can successfully attack mature, healthy tissues since invasion of callus in late atuumn has been observed in numerous older cankers, natural and artificial, during the present investigations. Consequently, during the period of active growth when callus is being rapidly formed, the canker decreases in size but the fungus is able to advance in the underlying woody tissues, hampered, it is true, by the presence of wound gum in the vessels and at times by temperatures adversely high (54). Now, if a freshly made wound is inoculated early in the growing season and conditions are suitable, *V. cincta* is sometimes able to invade the wood, although there may be little external evidence of infection at the time. Soon the growth of the fungus is retarded both by the deposition of wound gum in the host and by rising temperatures, while the development of the callus reduces the size of the wound. If, however, the fungus has succeeded in establishing itself, it invades the callus during the subsequent dormant season and a canker makes its appearance a year after infection actually occurred (Series 17, 18, 20, 21, 38, Figs. 2 and 3). The spring of 1932 was evidently favorable for infections of this sort while that of 1933 was not. The underlying reason is obscure but the shape of the curves indicates that the healing of new wounds proceeded at a greater rate in the latter year (compare all sets of Series 15–20 with those of 36–42, Figs. 2 and 3). It is also probable that the extreme heat combined with drought in the summer of 1933 did not permit appreciable growth of *V. cincta*, especially in recent inıections. It would appear that, in the spring, there is a delicate balance between conditions favoring the growth of the fungus and promoting infection and those favoring the host and tending to prevent infection. During June, July and August the balance is decidedly in favor of the host (Series 1, 22–25, 42–44, Figs. 2 and 3). Then, the wound-gum barrier, which at other times can be penetrated by *V. cincta* (54) appears to be effective against the slowly growing

pathogen which fails to secure a foothold. In September and early October, before leaf fall, when prevailing temperatures are closer to the optimum for the fungus, infection is again possible. At that time, the host is able to form only a small amount of callus but wound gum still appears, so that the spread of the fungus through the tissues is less rapid than when infection occurs after leaf fall when the production of wound gum is negligible (54), and if low temperature is not a limiting factor (compare Series 2–4, 26, 27 with Series 5, 6 and 7, Figs. 2 and 3).

In those series where partial healing of the lesions during the growing season alternated with further enlargement during the dormant season (Series 2–4, 11–18, 20, 26–31 etc., Figs. 2 and 3), only a few of the inoculated branches became girdled. However, where the majority were killed by *V. cincta* (Series 5–8, 15, 32, Figs. 2 and 3) a progressive increase in the size of lesions was observed throughout the year, although the rate of increase was usually less during the growing season than during the dormant season. In many instances (*e.g.*, Series 8, Fig. 2) the canker spread down the girdled inoculated branch and produced a lesion on one side of the parent branch (Plate II, Fig. 1). Girdling was determined partly by the size of the branch involved and partly by the season during which infection occurred.

V. leucostoma

The curves (Figs. 2 and 3) illustrating the behavior of wounds inoculated with *V. leucostoma* are remarkably uniform in general character, and with the exception of greater initial increase in size in several instances, are very similar to corresponding curves for check wounds, (see also Plate II, Figs. 3, 4, 7 and 8). This may be taken as evidence that *V. leucostoma* is comparatively innocuous, although the initial spread suggests that this organism may be a mild irritant. Indeed, this fungus has been re-isolated six to eight months after inoculation from wounds showing little, if any, necrosis. It is true that a number of active cankers have appeared, but in any set except Series 45 (see Fig. 1) at least 60% of the wounds remained healthy (see also Figs. 2 and 3, Series 6, 24, 25, 26 where the dotted line represents the average of both cankered and healthy wounds and the solid lines, healthy wounds only). Moreover, in 62% of the sets no cankers were formed. Consequently, even conceding that *V. leucostoma* gave rise to all the cankers that did appear, which was not the case since some of them at least were proved by isolations to be due to sporadic infection by *V. cincta*, the ravages of the former organism cannot be considered as being very serious.

The evidence as presented above is regarded as sufficient to warrant the view that *V. leucostoma* is not capable of acting as a primary parasite causing peach canker, but not to eliminate altogether the possibility that *V. leucostoma* may, under other circumstances, infect the peach and become a factor in the production of cankers. The work of McCubbin (35) and Togashi (51) suggests that, given tissue in which it can become established as a saprophyte, this organism may subsequently become an active parasite and invade healthy

tissues. As *V. leucostoma* has been isolated from peach, and its fruiting bodies occur thereon, it can evidently live on that host. However, the fact remains that as a primary cause of canker it is of much less significance than *V. cincta*.

S. fructicola

The parasitism exhibited by *Sclerotinia fructicola* falls into a category quite distinct from that of *V. cincta*. Unlike the latter organism, *S. fructicola* attacks the bark and wood of peach most readily during the growing season and its spread through infected tissues is very rapid. Fom May to September, this organism accomplishes its work within a period of three weeks, at most, after inoculation. This period corresponds with the time required by the host to lay down a wound periderm and to produce a wound-gum barrier (54), both of which seem adequate to delimit the fungus. After the cessation of the activity of the fungus the history of the lesions was identical with that of the checks, and in many cases (Series 16-24, 35-43, Figs. 2 and 3) considerable decrease in size occurred before the onset of the first dormant season. From October to April, when the host is inactive, *S. fructicola* seems to be able to spread through the host tissue at a rate limited mainly by the prevailing temperature. This accounts for the large amount of necrosis in Series 4 and 5 in the fall of 1931 (see Fig. 2 and Plate II, Figs. 5 and 6) when temperatures were relatively high throughout November. In the fall of 1932 (Series 27 and 28, Fig. 3) low temperatures after leaf fall evidently restricted infection by the fungus (Plate III, Fig. 5).

While temperature may sometimes be the limiting factor, it is not the only one which determines the rate of spread of *S. fructicola*. There is some evidence that humidity plays an important part in this connection. For example, in Series 17 (Fig. 2), the relative humidity was comparatively high (mean 82%) for the first day or so after inoculation, but the mean temperature was below 40° F. and the sky overcast so that branch temperature would approximate air temperature (51). Then when the temperature rose to the point where the fungus could grow, the humidity dropped (mean 59%). Later, when neither factor was limiting, periderm formation and wound-gum production had advanced sufficiently to delay the progress of the pathogen. Likewise in Series 19 (Fig. 2), while temperatures were favorable, humidity was low (mean 57%) and necrosis was slight. In Series 18, 20 and 21 (Fig. 2), however, where necrosis was severe, the mean relative humidity for the first ten days in each case was 72–73% and the temperature well above 45° F., except for the first three days in Series 18. In Series 16 (Fig. 2), although the mean temperature was for the most part adversely low, there was a period of about 18 hours during the second day when both temperature and humidity were favorable and the fungus evidently was able to gain entry. The absolute figures for relative humidity as given above are not in themselves especially significant, but they serve to indicate periods of drought or of dampness, confirmed by the continuous records of humidity. In the spring months, the succulent nature of the actively growing tissues would seem to be conducive to the growth of the fungus, once infection had occurred. In the height of

the summer, when tissues were firmer and branch temperatures higher and probably above the optimum for *S. fructicola*, especially on sunny days, there was less necrosis. This unfavorable set of conditions was in all probability supplemented also by the extreme diurnal fluctuations in humidity characteristic of summer weather. In any case in series inoculated during the summer months of 1932 and 1933, the least necrosis was coincidental with the highest mean temperature for the critical 10-day period following inoculation (Series 23 and 43, Figs. 2 and 3). In Series 44 the amount of damage was also small, but here a rainfall of one and one-half inches during the first 12 hours may conceivably have washed off much of the inoculum. Again, during the winter of 1932–33 the open wounds increased in size to a much less extent than the slits. It seems reasonable to suppose that this difference in behavior was due in large measure to a greater tendency on the part of the larger wounds to dry out.

The percentage of active cankers appearing after inoculation with *S. fructicola* (4.6%) was somewhat lower than in the case of *V. leucostoma* (6.8%) and slightly greater than in the case of checks (2%). These differences are scarcely significant except for the suggestion that delay in healing and the presence of necrotic tissue may provide more opportunity for natural infection with canker-producing organisms. There is no doubt that the active cankers which developed both in the series involving *S. fructicola* and in the checks were due to contamination, as valsoid pycnidia were almost invariably present. Here also re-isolations yielded *Valsa cincta*. Although in Series 45 (Fig. 1) *S. fructicola* was credited with 100% active cankers in the spring of 1934, this was undoubtedly due to the late date at which infection took place in 1933. However, according to the criteria the expectation is that the future history of this series will not be different from that of earlier ones.

There is no disputing the fact that some cankers on the peach, in their incipient stages, may be caused by infection with *S. fructicola*, but these experiments clearly demonstrate the invalidity of the term "brown rot cankers" as applied to peach cankers, especially if they are perennial. In nature, *S. fructicola* gains access to the twig, if not solely at least chiefly, through blighted blossoms and rotting fruit, but since the majority of cankers are found to originate at other points (55), then *S. fructicola* can be regarded as responsible for only a relatively small number of cankers. Undoubtedly the lesions initiated by *S. fructicola* are as subject to infection with canker-producing organisms, for example *Valsa cincta*, as any other sort of wound, but unless such infection occurs they are not likely to become typical peach cankers.

Checks

In considering the check wounds, two or three points of interest may be noted. In general, the growth of callus seems to be somewhat slower in wounds made early in the dormant season than in those made in spring. The exposed tissues of the former tend to dry out during the winter months as the slight enlargement of such wounds indicates, while in the latter the

healing processes become operative very soon after wounding. In most instances, also, the rate of closure was greater during the first growing season after wounding, during which time the wounds on the larger, more vigorously growing branches became almost, if not completely, healed. As a rule, the growth of callus was least in the direction parallel to the long axis of the branch and greatest at right angles, that is to say, as the wounds were overgrown by the callus developing along their sides rather than at their ends, decrease in length was achieved largely by the meeting of the lateral calli at the ends of the wounds. Consequently, large decreases during the second growing season (as in *V. cincta* 22, 23, 40, 42, 43; *S. fructicola* 27-29, 36, 42-44, and checks 20, 22 and 24, Figs. 2 and 3) do not denote rapid healing at that time so much as the meeting of lateral calli in wounds which were almost closed and therefore long and very narrow at the end of the first growing season. The callus development in wounds on smaller branches varied with the growth rate of the branch, so that in some cases, where the growth of the branch was negligible, little change occurred in the size of the wound. Such cases became apparent as a horizontal tendency in the curves, in the portion covering the third growing season (Series 1–24, Figs. 2 and 3).

To conclude, convincing evidence has been obtained that, in so far as peach canker is concerned, the fungus herein designated as *Valsa cincta* is in itself quite capable of acting as a primary causal organism infecting fresh wounds, as well as a successor to lesion-forming pathogens such as *Sclerotinia fructicola*. In either case, it is the important factor in the production of perennial cankers. *V. leucostoma*, on the other hand, is relatively unimportant as a primary parasite, whatever role it may assume under other circumstances in the later stages of the disease. However, it should be emphasized that, while there is no doubt of the major significance of *V. cincta* in the Niagara Peninsula of Ontario, no claim is made that this organism is the only pathogen concerned in the formation of perennial cankers on the peach.

Acknowledgment

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References

1. ADERHOLD, R. Ueber das Kirschbaumsterben am Rhein, seine Ursachen und seine Behandlung. Arb. Biol. Land-Forstw. 3 : 309-363. 1903.
2. BERKELEY, G. H. Report of the Dominion Botanist, Can. Dept. of Agr., for 1926 : 59-102. 1927.
3. BOYLE, C., MURPHY, M. and CUMMINS, H. A. "Blossom wilt" of apple trees and "wither tip" of plum trees, with special reference to two biologic forms of *Monilia cinerea* Bon. Sci. Proc. Roy. Dublin Soc. n.s. 19 : 63-76. 1928.
4. BROOKS, F. T. and MOORE, W. C. Silver leaf disease, V. J. Pom. Hort. Sci. 5 : 61-97. 1926.
5. CAESAR, L. A. Peach diseases. Ont. Dept. of Agr. Bull. 201 : 33-59. 1912.
6. CAYLEY, DOROTHY M. Fungi associated with "die back" on stone fruit trees, I. Ann. Appl. Biol. 10 : 253-275. 1923.
7. CHABROLIN, C. Les dépérissements de l'abricotier. Compt. rend. acad. agric. France, 15 : 583-588. 1929.

8. CHABROLIN, C. Notes et observations relatives aux dépérissements de l'abricotier. Ann. Epiphyties, 14 : 355-376. 1929.
9. CHESTER, F. D. The rot of peach and other stone fruits. Trans. Peninsula Hort. Soc. (Del.) 6 : 57-64. 1893.
10. COOK, M. T. The blossom blight of the peach. Phytopathology, 11 : 290-294. 1921.
11. CRISTINZIO, M. Osservazione su una Malattia del pesco. Rich. Divulg. Fitopat. per la Campania, ed il Mezzogiorno. 2 : 28-44. 1933.
12. CUNNINGHAM, G. H. Brown rot, *Sclerotinia cinerea* Schroet., its appearance, cause and control. New Zealand J. Agr. 25 : 83-93. 1922.
13. CURZI, M. Una gravia infezione da "Phytophthora" dei peschi. Rend. accad. Lincei. 19, ser. VI, 817-820. 1934.
14. CZARNECKI, HELEN. Studies on the so-called black-heart disease of the apricot. Phytopathology, 13 : 216-224. 1923.
15. DOWSON, W. J. The die back of apricots. Preliminary note. Tasmanian J. Agr. n.s. 2 : 165-166. 1931.
16. DUFRÉNOY, J. Dépérissement des arbres fruitiers dans le Massif Central. 13 pp. Clermont-Ferrand, G. Mont-Louis, 1927.
17. DUFRÉNOY, J. and M. L. Hadromycoses. Ann. Epiphyties, 13 : 195-212. 1927.
18. ELLIS, J. B. and EVERHART, B. M. The North American Pyrenomycetes. 1892.
19. FAES, H. and STAEHELIN, M. La maladie cryptogamique des abricotiers en Valais. Reprinted from Ann. Agr. Suisse, 1923, 22 pp. 1924.
20. FAES, H. and STAEHELIN, M. Les champignons et les insectes ennemis du cerisier. Ann. Agr. Suisse, 28 : 1-27. 1927.
21. FAES, H. and STAEHELIN, M. Quelques cas de dépérissement de l'abricotier en Valais. Pamph. Stat. Fed. d'Essais Vitic. Lausanne, 6 pp. 1931.
22. FAES, H., STAEHELIN, M. and BOVEY, P. La lutte contre les ennemis des arbres fruitiers, insectes et champignons, en 1932. Landw. Jahrb. Schweiz, 48 : 241-280. 1934.
23. FANT, G. W. The brown rot canker and twig blight of peach. Forty-third Ann. Rept. N.J. Agr. Expt. Sta. for year ending June 30, 1922 : 547-548. 1923.
24. FANT, G. W. The manner of infection of peach twigs by the brown rot fungus. Phytopathology 14 : 427-429. 1924.
25. FISCHER, E. Eine Krankheit von *Cydonia japonica*. Schweiz. Obst. Gartenbauzeit. 13 : 216-217. 1930.
26. HOMMA, Y. A canker disease of *Prunus mume* and *P. persica* caused by a species of *Camarosporium*. Bot. Mag. (Tokyo) 41 : 541-546. 1927.
27. JACKSON, H. S. Pear canker, *Monilia* sp. Ore. Agr. Exp. Sta. Crop and Pest Rept. 2 : 271-272. 1915.
28. JEHLE, R. A. The brown rot canker of the peach. Phytopathology, 3 : 105-110. 1913.
29. JOËSSEL, P. H. Quelques maladies du pêcher et de l'abricotier dans la region Rhodanienne. Prog. Agr. Vit. 90 : 350-353; 370-374. 1928.
30. JOËSSEL, P. H. and BORDAS, J. Recherches sur les dépérissements de l'abricotier, dans la vallée du Rhone (1927–30). Ann. Epiphyties, 18 : 325-359. 1931.
31. KOCH, L. W. Investigations on the black knot of plums and cherries. IV. Studies in pathogenicity and pathological histology. Sci. Agr. 15 : 729-744. 1935.
32. LEONIAN, L. H. The physiology of perithecial and pycnidial formation in *Valsa leucostoma*. Phytopathology, 13 : 257-272. 1923.
33. MCCLINTOCK, J. A. Peach disease control. Ga. Agr. Expt. Sta. Bull. 139 : 1-30. 1921.
34. MCCLINTOCK, J. A. The brown rot situation in 1929. Am. Fr. Growers' Mag. 50 : 11, 30. 1930.
35. MCCUBBIN, W. A. Peach canker. Dom. of Can. Dept. Agr. Bull. 37 (2nd series) : 1-20. 1918.
36. MANNS, T. F. and ADAMS, J. F. Diseases of peaches and their control. Brown rot. Del. Agr. Expt. Sta. Bull. 133 : 27-28 1922.
37. MIX, A. J. A blight of flowering almond, (*Prunus glandulosa* Thunb.). Phytopathology, 20 : 265. 1930.
38. MIX, A. J. Brown rot, leaf and twig blight following peach leaf curl. Phytopathology, 20 : 265-266. 1930.
39. OVERHOLTS, L. O. Mycological notes for 1930-32. Mycologia, 25 : 418-430. 1933.
40. PFEIFFER. Von den Sorgen der Gummierkrankung unserer Kirschbäume. Z. Obst.- Wein.- und Gartenbau, 58 : 203-205. 1932.

41. RIVES, L. Sur les causes du dépérissement de l'abricotier par apoplexie. Compt. rend. Acad. d'Agric. France, 15 : 77-83. 1929.

42. RIVES, L. Nouvelles observations sur l'apoplexie de l'abricotier et de divers arbres fruitiers. Prog. Agr. et Vit. 95 : 88-91. 1931.

43. ROBERTS, J. W. and DUNEGAN, J. C. Peach brown rot. U.S. Dept. Agr., Tech. Bull. 328 : 1-59. 1932.

44. ROLFS, F. M. Winter killing of twigs, cankers and sun scald of peach trees. Mo. Sta. Fruit Expt. Sta. Bull. 17 : 1-101. 1910.

45. SACCARDO, P. A. Sylloge Fungorum. Vol. 1, pp. 139; 142. 1882.

46. SCHILBERSZKY, K. Ueber die Ursachen der Apoplexie bei den Steinobstbäumen. Ang. Bot. 14 : 536-551. 1932.

47. SCHILBERSZKY, K. Ueber die Ursachen der Apoplexie bei den Steinobstbäumen, II. Ang. Bot. 15 : 106-122. 1933.

48. SMITH, E. F. Peach rot and peach blight, (*Monilia fructigena* Persoon.). J. Mycology, 5 : 123-134. 1889.

49. SMITH, E. F. Peach blight, *Monilia fructigena* Persoon. J. Mycology, 7 : 36-38. 1891.

50. TOGASHI, K. Morphological studies of *Leucostoma leucostoma* and *Valsa japonica*, the causal fungi of canker or die-back disease of peach trees. Bull. Imp. Coll. Agr. For. Morioka, Japan, 14 : 1-50. 1930.

51. TOGASHI, K. Studies in the pathology of peach canker. Bull. Imp. Coll. Agr. For. Morioka, Japan, 15 : 1-178. 1931.

52. VAN DER MEER, J. H. H. Verticillium wilt of herbaceous and woody plants. Meded. Landbouwhoogeschool, Wageningen, 28 : 1-82. 1925.

53. WALTON, R. C. and BABCOCK, D. C. The parasitism of *Valsa leucostoma* (Abs.). Phytopathology, 6 : 112-113. 1916.

54. WILLISON, R. S. Wound-gum in peaches and grapes. Its relation to the invasion of fungus wound-parasites. Sci. Agr. 12 : 402-419; 484-505. 1932.

55. WILLISON, R. S. Peach canker investigations, I. Some notes on incidence, contributing factors and control measures. Sci. Agr. 14 : 32-47. 1933.

56. WILSON, E. E. Bacterial canker of stone fruit trees in California. Hilgardia, 8 : 83-123. 1933.

57. WORMALD, H. The *Cytospora* disease of the cherry. Rep. on Econ. Myc. 1912, S.E. Agr. Coll., Wye, Kent, England, 45-71. 1912.

58. WORMALD, H. A blossom wilt and canker of apple trees. Ann. Appl. Biol. 3 : 159-204. 1917.

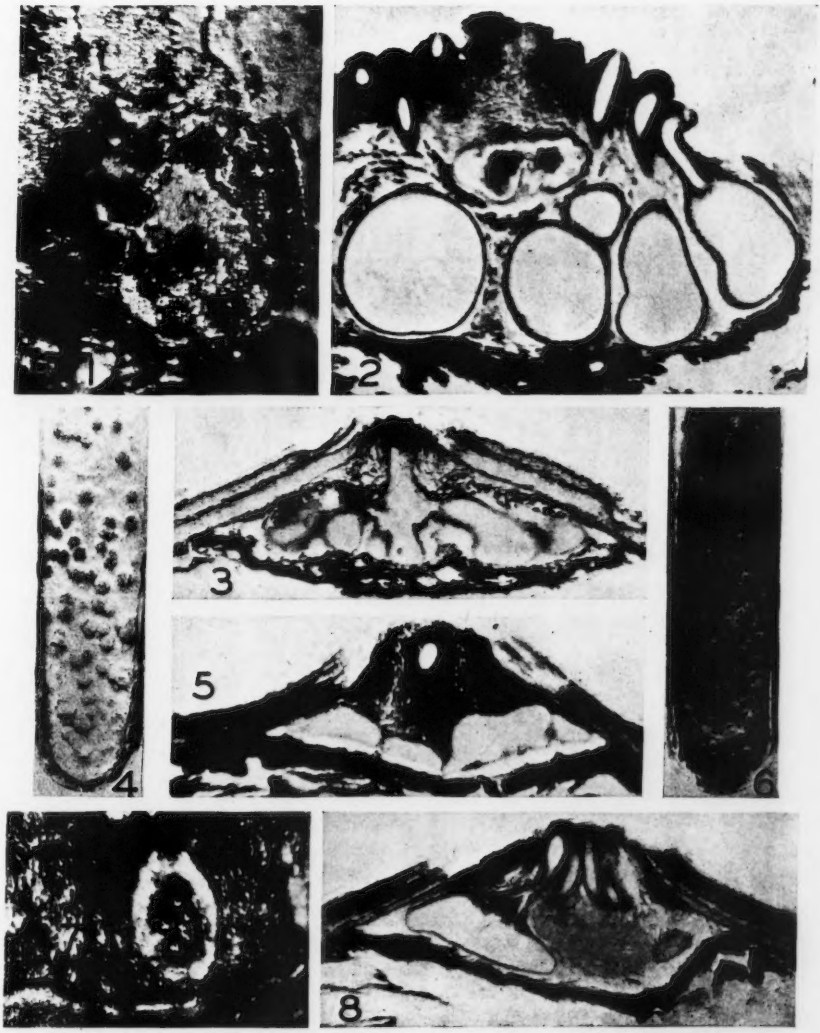
59. WORMALD, H. A 'wither tip' of plum trees. Ann. Appl. Biol. 5 : 28–59. 1918.

60. WORMALD, H. Further studies of the "brown rot" fungi, I. A shoot wilt and canker of plum trees caused by *Sclerotinia cinerea*. Ann. Botany, 36 : 305-320. 1922.

61. WORMALD, H. Further studies of the "brown rot" fungi, IV. *Sclerotinia fructigena* as the cause of an apple canker. Trans. Brit. Myc. Soc. 15 : 102-107. 1930.

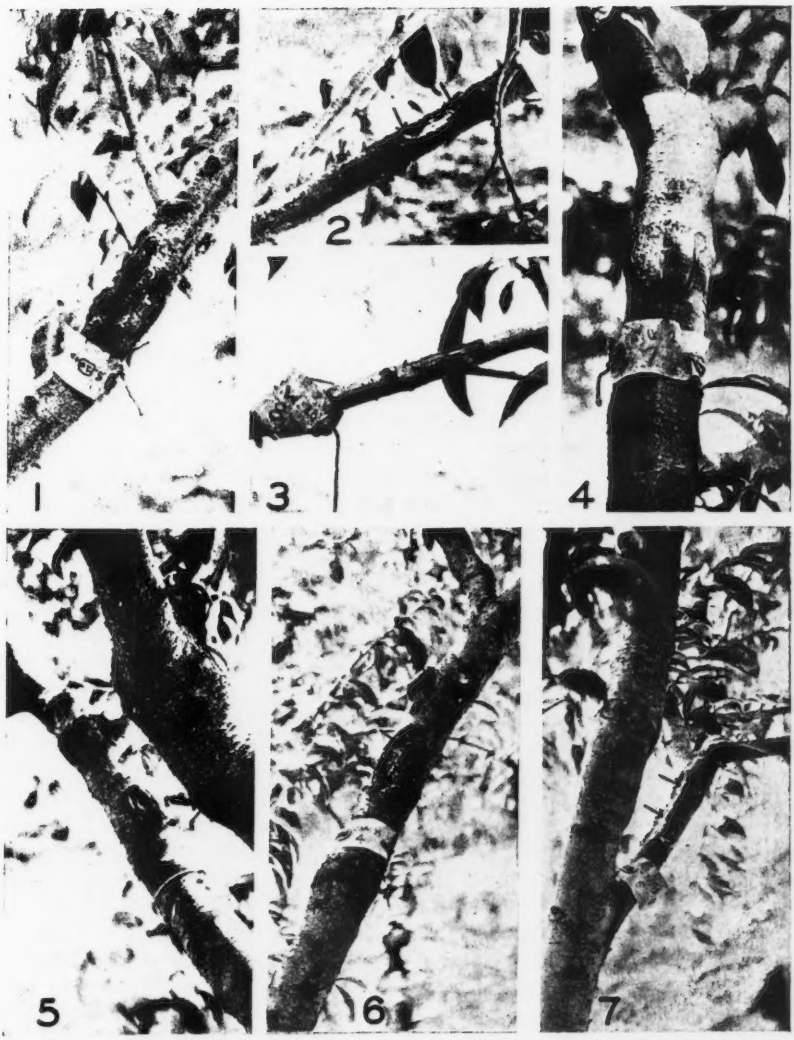
62. WORMALD, H. Further studies of the "brown rot" fungi. V. Brown rot blossom wilt of pear trees. Ann. Botany, 44 : 965-974. 1930.

63. WORMALD, H. Bacterial diseases of stone fruit trees in Britain. III. The symptoms of bacterial canker in plum trees. J. Pom. Hort. Sci. 9 : 239-256. 1931.



FIGS 1 TO 4. *Valsa cincta.* FIGS. 5 TO 8. *Valsa leucostoma.* FIGS. 1 AND 7. *Erumpent discs of perithecial stromata.* FIGS. 2 AND 8. *Median sections through perithecial stromata.* FIGS. 3 AND 5. *Median sections through pycnidial stromata.* FIGS. 4 AND 6. *Cultures on potato-dextrose agar. Magnification of photomicrographs approximately 40 diameters. Cultures natural size.*

Typical wounds from series 5, made and inoculated October 19, 1931; photographed June 19, 1933. FIGS. 1 AND 2. *With Valsa cincta, three badly cankered lesions.* FIGS. 3 AND 4. *With V. leucostoma, one healed and two still open but not cankered.* FIGS. 5 AND 6. *With S. fructicola. Note extent of original necrosis, followed by healing.* FIGS. 7 AND 8. *Checks, one healed and one open wound. Arrows, where present, indicate original size of wound.*



Typical wounds, photographed June 19, 1933, comparing effects of inoculation in growing and in dormant seasons. FIGS. 1 AND 2. *S. fructicola, Series 20, inoculated May 30, 1932.* FIGS. 3 AND 4. *V. cincta, Series 23, inoculated July 11, 1932.* FIG. 5. *S. fructicola, Series 33, inoculated Dec. 3, 1932.* FIGS. 6 AND 7. *V. cincta, Series 33, also inoculated Dec. 3, 1932. Note: necrosis occurred in 1, 2, 6 and 7 but not in 3, 4 and 5. Arrows where present, indicate original size of wound.*

FLAX STUDIES

I. THE RELATION BETWEEN WEIGHT PER MEASURED BUSHEL, WEIGHT PER THOUSAND KERNELS AND OIL CONTENT OF FLAXSEED[1]

By W. F. Geddes[2] and F. H. Lehberg[3]

Abstract

Determinations of test weight per bushel, weight per 1000 kernels and oil content made on 146 samples of Western Canadian flaxseed, 119 of which graded No. 1 C.W., 16 No. 2 C.W. and 11 No. 3 C.W., revealed that while the grades were differentiated in regard to test weight per bushel and weight per 1000 kernels, the mean oil contents for the three grades were not significantly different. Test weight per bushel is of little significance as an index of oil content. While the degree of association of weight per thousand kernels with oil content is somewhat closer, it is not sufficiently high to permit satisfactory prediction of this variable.

Introduction

In the inspection and grading of the cereal grains, the primary object is to classify the product into groups of differing quality in regard to its suitability for the particular purpose which it is tended to serve. Since chemical determinations are in most instances impractical owing to the time, the cost, and the volume of work required, it is necessary in practical grain grading to utilize certain physical characteristics which can be readily determined or estimated. In the grading of flaxseed in Canada and the United States, test weight per bushel, content of damaged seeds, presence of foreign material, and moisture content are the principal factors employed. Since linseed oil is the most valuable product obtained from flaxseed, the grading factors should reflect the quantity and quality of oil in the seed. In 1927 Coleman and Fellows (1) reported the results of a study of the relation between oil content of flaxseed and such variables as test weight per bushel, size of seed, moisture content, damaged and foreign seed content, and found that the oil content was not closely related to the percentage of certain types of damaged kernels nor to test weight per bushel. These workers reported, as a result of an examination of some 2000 samples of flaxseed, that test weight per bushel is of little significance as an index of oil content, the mean oil contents for samples weighing from 47 to 53 lb. per bushel being practically equal, although there appeared to be a tendency for samples of high test weight to contain less oil than those of lower weight.

Preliminary experiments undertaken in this laboratory indicated that the weight per 1000 kernels is more closely related to the oil content of flaxseed than test weight per bushel, which led to the study of the relation between weight per thousand kernels, test weight per bushel and oil content of flaxseed reported in this paper.

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Experimental

The experimental material consisted of 146 samples of Western Canadian flaxseed taken from cars sampled in the Winnipeg Inspection Division, during the past two crop years. The production of flaxseed in Western Canada has fallen to such a low level that it was found difficult to secure many samples, particularly in the grades lower than No. 1 C.W. Of the total of 146 samples collected, 119 graded No. 1 C.W., 16 No. 2 C.W. and 11 No. 3 C.W. However, these represented approximately 40% of the combined Western Canadian crop inspected at Winnipeg for the two crop years, and hence may be regarded as fairly representative.

Foreign material was removed from the samples by means of a Perkins flax cleaner, and the cleaned seed employed for the analytical studies. Test weight per bushel was determined in duplicate by means of an Imperial pint or ½ pint bucket, a Cox funnel being used, and the seed weighed with a weight per bushel balance. As the samples had been stored in the laboratory for several weeks, their moisture contents were practically identical (approximately 5.0%). In any event, Coleman and Fellows (1), have shown that, as compared with other cereals, variations in the moisture content of flaxseed have little influence on test weight per bushel.

TABLE I

STATISTICAL CONSTANTS

Grade No.	No. of samples	Means			Standard deviations			Coefficients of variability		
		Wt. per bushel, (x) lb.	Wt. per 1000 kernels, (y) gm.	Oil cont. (s) %	Wt. per bushel, (x) lb.	Wt. per 1000 kernels, (y) gm.	Oil cont. (s) %	Wt. per bushel, (x) %	Wt. per 1000 kernels, (y) %	Oil cont. (s) %
1 C.W.	119	54.52	5.287	40.76	1.29	0.950	1.52	2.4	18.0	3.7
2 C.W.	16	50.04	4.817	41.47	2.23	0.436	1.52	4.4	8.8	3.7
3 C.W.	11	48.50	4.714	40.65	1.78	0.674	2.14	3.7	14.3	5.3
All grades	146	53.58	5.192	40.83	2.48	0.912	1.58	4.6	17.6	3.9
Variance between grades (F)		14.00	3.77	1.51						

Variance within grades at 5% pt. F. = 3.06

Simple correlation coefficients

Correlation		1 C.W.	2 C.W.	3 C.W.	All grades
Wt. per bu. (x) × oil content (s)	r_{xs}	−.225	.001	.326	−.126
Wt. per 1000 kernels (y) × oil content (s)	r_{ys}	.487	.327	.672	.444
Wt. per bu. (x) × wt. per 1000 kernels (y)	r_{xy}	−.157	−.135	.040	.106
At 5% pt. r =		.182	.497	.602	.164

In determining weight per 1000 kernels, 500 kernels were counted out at random, in duplicate, weighed to ± 0.01 gm. and the results computed to a dry matter basis. Oil content was determined in duplicate by the ethyl ether extraction method outlined in the second paper of this series (2).

The statistical constants computed on the means of the duplicate values for weight per bushel, weight per 1000 kernels and oil content, recorded in Table I, summarize the results obtained. From the mean values of these variables and the "F" values for the ratios of the variances contributed by the differences between grades to the corresponding variances between the samples within the grades, it will be noted that, while there is a significant decrease in test weight per bushel and weight per 1000 kernels with a decrease in grade, the mean values for oil content are not significantly different. The simple correlations reveal that in grade No. 1 C.W. there is a slight tendency for high oil content to be associated with low test weight per bushel, although such a relation does not exist for the other two grades. On the other hand, weight per 1000 kernels is positively correlated with oil content, and the correlations are of a higher order of magnitude than those involving test weight per bushel. However, the degree of association is not sufficiently high to permit a satisfactory prediction of oil content by its use; moreover, the determination is laborious and for this reason alone, weight per 1000 kernels is unsuitable as a grading factor.

The results on this limited series of samples of Canadian flaxseed fully confirm the observations of Coleman and Fellows (1) that weight per bushel is of little significance as an index of oil content, and indicate the necessity of developing a convenient and rapid method for estimating oil content if the grading of flax is to be placed on a scientific basis.

References

1. Coleman, D. A. and Fellows, H. C. U.S. Dept. Agr. Bull. 1471. 1927.
2. Geddes, W. F. and Lehberg, F. H. Can. J. Research, C, 14 : 48-61. 1936.

FLAX STUDIES

II. AN IMPROVED REFRACTOMETRIC METHOD FOR ESTIMATING THE OIL CONTENT OF FLAXSEED[1]

By W. F. Geddes[2] and F. H. Lehberg[3]

Abstract

The method proposed by Coleman and Fellows, based upon the change in refractive index of halowax, an impure substituted monochlornaphthalene, on dilution with linseed oil extracted from the ground sample, is improved by drying the sample before extraction, grinding in a Hobart mill, filtration for 15 minutes, and the use of a special refractometer with illumination by a 40 watt light. The use of 4 cc. of solvent and extraction at 70° C. was confirmed. The correlation between halowax extract scale reading and oil content as determined by ethyl ether extraction was 0.95; the standard error of prediction of oil content by the refractometric method was 0.59%. The refractometer readings were not appreciably affected by variations in the refractive index of linseed oil as determined on the ether extract. The correlation between the refractive index and the iodine value of the ether extract was 0.70 with a standard error of prediction of 3.1 units.

Further improvement is obtained by the use of a solvent consisting of approximately 50% by volume of halowax and α-bromnaphthalene which gives a standard error of prediction of 0.39%. By slight changes in the proportions, the refractive index of this solvent may be adjusted to a constant value, permitting the use of a permanent conversion chart. Addition of anhydrous sodium sulphate during the extraction obviates the necessity of preliminary drying. With the modifications indicated, the method is suitable for routine determinations on large numbers of samples.

Introduction

In the first paper of this series, Geddes and Lehberg (4) reported that neither test weight per bushel nor weight per thousand kernels was of any practical utility as a measure of the oil content of Canadian flaxseed. The conventional chemical methods for determining the oil content of flaxseed, involving extraction with solvents, as pointed out in the previous paper, are not applicable for routine grading purposes and the experiments reported here were accordingly undertaken to investigate the possibility of a rapid optical method.

The use of an optical method for the measurement of the oil content of oil-bearing seeds was first proposed in 1920 by Wesson (5), who developed a refractometric method for cottonseed meal and cottonseed meats. This method is based upon the change in the refractive index of a solvent known commercially as halowax, an impure substituted monochlornaphthalene, as it became diluted with cottonseed oil extracted from the sample. This solvent is well suited to a test of this nature, since it is essential, in order to secure the greatest accuracy, that the indices of refraction of the vegetable oil and the solvent should differ widely. Halowax oil has a refractive index

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of 1.635 at 25° C., which is considerably higher than the refractive indices of the vegetable oils. Other fundamental requirements of a suitable solvent which are possessed by halowax are that it should be non-volatile, non-inflammable, inexpensive, and possess a low coefficient of expansion.

Working with cottonseed meal, a material of low oil content, Wesson found his method gave values in good agreement with those obtained by the usual ether extraction method but in the cottonseed meats, which are of higher oil content, the agreement was not so satisfactory. Coleman and Fellows (1, 2) accordingly made a study of the method and introduced certain modifications in technique to render the test applicable to the estimation of the oil content of flaxseed and other oil-bearing seeds. Their technique for flaxseed consisted of triturating 2 gm. of the finely ground sample in a 3-in. porcelain mortar previously heated to 70° C. with 4 cc. halowax and 1 to 2 gm. of fine sea sand for at least 2 min., filtering and determining the refractive index of the filtrate at 25° C. with an Abbé refractometer, an instrument which could be read to the fifth place. The refractive index found was converted to oil content by means of a conversion table prepared by adding definite amounts of a petroleum ether extract of flaxseed to a known quantity of halowax oil and noting the refractive indices of the solutions. The relation between the change in the refractive index of halowax oil and the linseed oil content of the solution was found to be linear, each 1% of linseed oil present reducing the refractive index of the solvent by 0.00191 units. The method was applied to the analysis of 120 samples of flaxseed and the results compared with those obtained by the A.O.A.C. petroleum ether extraction method. The greatest individual variation found was 0.33%, while the oil content of 80.8% of the samples tested agreed within ±0.2%, and 45.8% within ±0.1% by the two methods. The average deviation between the results by the two methods has been computed by the present authors and found to be 0.12%, while the standard deviation of the differences was 0.08%.

In preliminary tests by this laboratory, the application of the refractometric method, as described by Coleman and Fellows (2), to the estimation of the oil content of Western Canadian flaxseed, did not give results in satisfactory agreement with the ether extraction method. In these studies, the samples were weighed out on a dry matter basis and the presence of varying amounts of moisture was found to introduce serious errors due to cloudy filtrates, which made reading difficult, and probably also to interference with the efficiency of extraction of the linseed oil by the solvent. Considerable improvement was obtained by removal of moisture before trituration with halowax, thus eliminating the formation of emulsions, but it seemed essential that a refractometer capable of being read accurately to the fifth decimal place be employed in order to bring the accuracy of the method within desirable limits. Moreover, the method employed by Coleman and Fellows in preparing the conversion table is based on the assumption that the linseed oil extracted by halowax corresponds in refractive index to that obtained with petroleum ether. While it is presumed that the petroleum ether extract used

was a composite from several samples, thus taking into consideration variations in the refractive indices of linseed oil from different samples, it would seem preferable to determine the oil content and refractive index of a halowax extract of a series of samples and prepare a conversion table on the basis of the regression of oil content on refractive index.

In view of these considerations, the study reported here was undertaken with the object of developing a more satisfactory refractometric method for estimating the oil content of Canadian flaxseed.

Experimental

For the purpose of this study a special refractometer, constructed by the Zeiss Company according to specifications supplied by the laboratory, was obtained. This instrument, shown in Fig. 1, combines the dipping tube and eye piece with the Abbé water-jacketed head. Three prisms are provided, the measuring range of each covering individually the refractive index limits of linseed oil, halowax, and the linseed-oil–solvent mixtures as follows:

Scale Reading	*Refractive Index*
−5.0 to +105.0	1.46514 to 1.48986
−5.0 to +105.0	1.62191 to 1.65303
−5.0 to +105.0	1.62438 to 1.58908

By thus limiting the range of each prism head, a greater degree of accuracy is obtainable; the accuracy with which the arbitrary scale values of the instrument may be read corresponds to a determination of refractive index to the fifth decimal place, and by interpolation to the sixth place.

Preparatory to the analysis of a series of samples of flaxseed, it seemed advisable first to study the influence of such variables as fineness of grind, flaxseed-solvent ratio, temperature and time of extraction, and light source, on the results by the refractometric method.

In regard to light source, daylight did not give a sufficiently sharp line of demarcation to permit accurate readings being made, while sodium light and that given by a 40 watt, inside frosted, incandescent, electric light bulb were equally satisfactory; for practical reasons the latter was employed.

Tests conducted with samples ground to different degress of fineness showed, as pointed out by Coleman and Fellows (1, 2), that uniformity in grinding is essential for consistent results. These workers recommend grinding in an experimental flour mill, fitted with 6 × 6 in. rolls, corrugated 40 to the inch and operated at a differential of $1\frac{1}{2}$ to 1. In our hands, this method did not give a uniformly ground product, the pulp tending to remain in the corrugations and to accumulate on the housing beneath the front roll, rendering cleaning of the mill between samples tedious and difficult. As a result of pressure, the pulp which accumulated in the corrugations is very oily and the portions of this which become detached do not mix readily with the more floury portions of the pulp. The grind that could be obtained in the most uniform manner without any appreciable day-to-day variation was a fine pulp grind obtained

on the Hobart Model 6 burr mill (equipped with stationary burr No. 4317, No. 2 R.N. Sta. MCH No. 6 Ex PG and rotary burr No. 4318 No. 2 P.H. Rot. MCH No. 6 Ex PG), with a setting of from 5.0 to 5.5.

Owing to the nature of the material, a measure of the fineness of grind, by means of sieving, cannot be obtained without first extracting the oil. The results of typical sieving tests on the residues after extraction with ethyl ether are shown in Table I.

TABLE I

TYPICAL SIEVING TEST ON ETHER-EXTRACTED FLAXSEED PULP

Flour silk No.	Meshes per square inch	Weight retained
0 XX	1444	6 – 10
2 XX	2916	30 – 33
4 XX	3844	5 – 6
Total		41 – 49%
Total passing through No. 4 XX =		51 – 59%

In the Coleman and Fellows method, 2 gm. of ground flaxseed is extracted with 4 cc. of halowax at 70° C. It seemed desirable, in studying the effect of variations in oil-solvent ratio, to conduct the extractions at room temperature as well as at 70° C. since, if satisfactory results could be obtained at the lower temperature, the test would be considerably simplified. Five samples of flaxseed, ranging in oil content from approximately 38% to 43% were extracted at 24° and 70° C. respectively, using 3, 4, 5 and 6 cc. halowax and 2 gm. moisture-free flax pulp. Quadruplicate extractions were made for each temperature and solvent-oil ratio and quadruplicate readings on each extract. A statistical study of the resulting data indicated that extraction at 70° C. gave a much better differentiation between samples than extraction at 20° C. Also 4 cc. solvent gave the greatest range between samples with the minimum variability within replicates, the results therefore confirming the conditions selected by Coleman and Fellows in regard to these particulars.

While variations in maceration time of from 1 to 4 min. were found to be without any significant influence on the refractive index readings, investigation showed that the first few drops of the filtrate were not representative of the extract, the best results being obtained with a filtration time of approximately 15 min., sufficient time also being allowed for the filtrate to acquire the temperature of the prism, *i.e.*, 25° C., before taking readings.

An essential requirement for accuracy and rapidity in conducting refractometer readings is the provision of a convenient and reliable constant temperature control. Early tests were made using the hanging wall-tank and gas-heated coil apparatus, but this method was abandoned owing to its lack of efficiency. The present equipment, as shown in Fig. 1, consists of a constant pressure tower fitted with a valve to regulate the flow of water through a water-cooled rheostat. By proper adjustment of the valve and the sliding contact of the rheostat the effluent can be easily held within $\pm 0.1°$ C., the variations normally being within $\pm 0.05°$ C.

On the basis of these preliminary experiments, the following method was employed for the purpose of studying the relation between oil content and refractive index of the halowax extract.

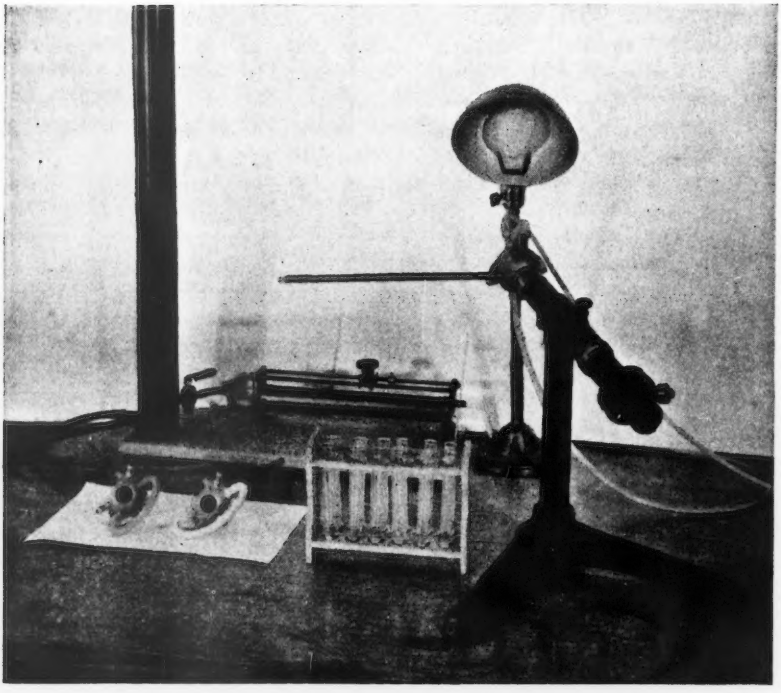

FIG. 1. *Refractometer and temperature control device.*

The flaxseed is ground to a fine pulp in the Hobart burr mill and then dried overnight in the vacuum oven at 98°–100° C.

Two grams of the dried material is accurately weighed and triturated for at least 1 min. in a mortar previously heated to 70° C. with 1 gm. of fine sand and 4 cc. halowax.

The mixture is filtered through a 70 mm. folded Prat-Dumas filter paper and the filtrate collected in a test tube.

After filtration for approximately 15 min. the refractive index of the filtrate is determined at 25° C., one minute being allowed for the extract to acquire the temperature of the prisms before taking the readings.

Triplicate halowax extractions with duplicate scale readings on each extraction were made and the mean values employed for the subsequent statistical studies reported here.

In regard to the determination of the oil content of flaxseed, there is apparently no standard method in general use, as indicated in the replies received to a questionnaire submitted to research and industrial laboratories early in 1934. Not only was there considerable variation in the type of extraction apparatus employed but the times of extraction and the solvents used differed

widely. For this reason it seems essential to describe in some detail the method employed in this laboratory. A 10 gm. sample of the flax pulp, ground in the Hobart burr mill as described for the refractometric studies, is dried overnight *in vacuo* at 98°–100° C. and extracted on an electrically heated water bath for 16 hr. with anhydrous alcohol-free and peroxide-free ethyl ether in a Soxhlet extractor, using Whatman double thickness extraction thimbles, and a siphoning rate of 1 per minute. The ether extract is filtered through a sintered glass filter and transferred directly to a tared 125 cc. Erlenmeyer flask by suction, the extraction flask being washed three times with fresh solvent. This procedure is necessary to remove traces of starch which are inevitably present in the extract. The excess ether is distilled off on a water bath maintained at approximately 70° C., the extract dried *in vacuo* for 3 hr. at 98°–100° C. at a pressure not exceeding 25 mm. mercury, and the oil content computed to a dry matter basis.

It has been found that increasing the time of extraction beyond 16 hr. does not increase the quantity of oil recovered. However, if the extracted sample is transferred to a mortar and re-ground with fine carborundum or sand and re-extracted with ethyl ether for a further 12 hr., a slight additional quantity of oil is obtained. The re-grinding increases the tendency for starch to be present in the extract and it is particularly necessary that the extract be filtered before evaporation. The quantity of oil recovered from a number of re-ground, previously extracted samples varied from 0.12 to 0.30% with an average of 0.16%. In view of the relative constancy of the additional oil and the inconvenience of re-extraction, single extractions only were made in the present study.

In order to determine whether variations in the refractive index of the ether extract had any appreciable influence on the corresponding values obtained on the halowax extract, refractometric scale readings were made on the ether extracts at 25° C. The iodine values of these extracts were also determined in duplicate according to Wijs' method.

Sixty-eight samples of flaxseed grown in different districts of Western Canada in the crop years of 1931, 1932 and 1933 and for the most part grading No. 1 C.W., were submitted to the analytical determinations outlined above and the statistical constants computed from the respective mean values obtained for the individual samples are given in Table II.

The close relation between the scale reading of halowax extracts and oil content, as determined by ether extraction, is shown by the high and significant correlation $r_{yz} = .948$. As $1 - r^2$ may be regarded as an estimate of the fraction of the total variance of halowax scale reading independent of oil content, 90% of the variance is attributable to the correlation. That the scale readings on halowax extracts are not appreciably influenced by variations in the refractive index of linseed oil, as determined on ethyl ether extracts, is shown by the partial correlation $r_{yz \cdot x} = .122$. This result is quite surprising and suggests that either the solvent actions of ethyl ether and halowax are dissimilar or that the refractive index of linseed oil is modified in an

TABLE II

STATISTICAL CONSTANTS FOR DATA ON OIL CONTENT, SCALE READING OF HALOWAX EXTRACT, IODINE VALUE AND SCALE READING OF ETHER EXTRACT

(N = 68)

Means, standard deviations and ranges

		Mean	S.D.	Range
Oil content, %	(x)	40.37	1.75	37.0 – 43.8
Halowax extract scale reading	(y)	45.63	3.62	38.5 – 51.7
Iodine value (Wijs)	(z)	184.38	4.36	174.6 – 196.5
Ether extract scale reading	(s)	40.55	4.43	33.4 – 64.0

Simple correlation coefficients

		r	t*
Oil content × halowax extract scale reading	r_{xy}	.948	24.0
Oil content × iodine value	r_{xz}	−.258	2.1
Oil content × ether extract scale reading	r_{xs}	.246	2.0
Halowax extract scale reading × iodine value	r_{yz}	−.241	2.0
Iodine value × ether extract scale reading	r_{zs}	−.700	8.0
Halowax extract scale reading × ether extract scale reading	r_{ys}	.271	2.3

*At 5% point t = 1.9
t = 1.0

First order partial correlation coefficients $r_{ys \cdot z}$ = .122

Regression coefficients

		B	t
Oil content on halowax extract scale reading	b_{xy}	.458	24.0
Iodine value on ether extract scale reading	b_{zs}	−.689	8.0

Linear regression equations

	Standard error of prediction
Estimation of oil content (x) from halowax scale reading (y) x = 19.471 + .458y	0.59%
Estimation of iodine value (z) from ether extract scale reading (s) z = 212.38 − .689s	3.12

entirely irregular manner by the laboratory manipulations involved in the ether extract determination. This point is now being investigated by comparing the refractive indices and iodine values of ether extracts of flaxseed with the corresponding values determined on linseed oil expressed by means of a Carver laboratory press.

Since the scale readings of halowax extracts depend to such a large extent on the oil content of flaxseed, they may be employed as an estimate of oil content by having recourse to the regression equation x = 19.471 + .458 y,

which gives the most probable value of x for determined values of y. The linearity of regression was tested by the analysis of variance procedure, (Fisher (3)) and the deviations from the regression line were not significant. Hence, the standard error of a single predicted observation is defined by $\sqrt{1 - r^2}\,\sigma x$, which in this instance equals 0.59%.

For routine work the estimation of iodine value from the refractive index of linseed oil would be of considerable utility. Accordingly, the regression of iodine value on scale reading of the ether extract was computed and is given in Table I. The relation between these variables was found to be linear, the standard error of a single predicted iodine value being ± 3.1.

It is of interest to note that there is a slight tendency for low iodine values to be associated with high oil content, as shown by the correlation $r_{xz} = -.258$.

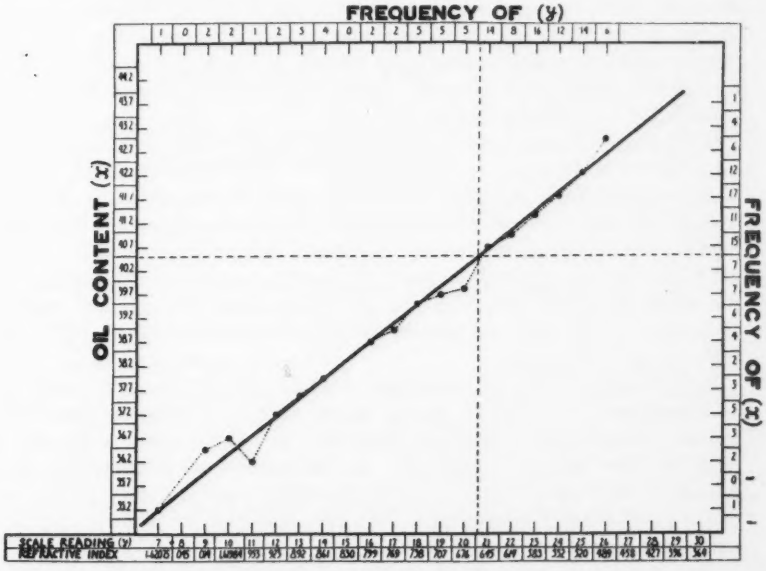

FIG. 2. *Regression of oil content of flaxseed on refractive index and scale reading of halowax-α-bromnaphthalene (50 : 50 by volume) extracts.*

The accuracy of prediction of the oil content of flaxseed by the refractometric method, using halowax as solvent, leaves much to be desired. Moreover, as this substance is impure, its refractive index is not constant throughout various shipments. This necessitates frequent revision of the conversion table. It was therefore decided to investigate the possibility of other available linseed oil solvents of satisfactory physical properties. Three solvents were considered and their characteristics and approximate costs as compared with halowax, are given in Table III.

TABLE III

Solvent	Refractive index, at 25° C.	Boiling point, °C.	Specific gravity	Approximate cost per 500 gm. $
Monochlornaphthalene (halowax)	1.635	246	1.368	0.75
α-methylnaphthalene	1.618	243	1.001	1.50
Tetra bromethane	1.638	151	2.92	3.00
α-bromnaphthalene	1.6558	281	1.487	3.00

The refractive index of α-methylnaphthalene is not sufficiently high to warrant consideration; also the practical grade is colored and the refined too high in cost. While tetrabromethane has a refractive index similar to halowax, the high specific gravity would render its cost excessive for routine testing. On the other hand, α-bromnaphthalene has a higher refractive index than that of monochlornaphthalene, thus making it a superior solvent since the greater the difference in refractive indices of the solvent and linseed oil, the greater the attainable accuracy.

Since the refractive index of bromnaphthalene fell beyond the measuring range of any of the prisms at hand, and also in view of the relatively high cost of this solvent, it was decided to investigate the utility of a mixture of approximately 50% by volume with halowax. The mixed solvent possesses the advantage that its refractive index can be adjusted to a constant value (1.64461 at 25° C.) by varying slightly the percentages of its components, thus eliminating the inconvenience of adjusting the conversion table for each new shipment, as is required for halowax, and making it possible to prepare a conversion table for permanent use.

Refractometric determinations were made on 106 samples of flaxseed, using as the solvent a mixture of approximately 50% by volume of α-bromnaphthalene and α-chlornaphthalene (halowax), and the method previously outlined for the halowax extraction. As before, the oil content was determined by ethyl ether extraction and the iodine number and refractive index of the ether extract determined. The data obtained are summarized by the statistical constants recorded in Table IV.

The correlation between oil content of flaxseed and scale reading of the mixed solvent extracts, $r_{xy} = .977$, is significantly higher than the corresponding correlation of .948 for the halowax data. As in the instance of the halowax extractions, the regression of oil content on scale reading was found to be linear. The regression coefficient $b_{xy} = 0.393$ is significantly lower than that for halowax ($b_{xy} = 0.458$) so that a unit change in scale reading corresponds to a smaller increment of change in oil content for the new solvent, thus increasing the accuracy of estimation, as shown by the lower standard error of prediction of $\pm 0.39\%$. The regression line is shown in Fig. 2 with the frequencies for each scale reading in 1-unit class intervals and the actual means and frequencies for oil content in 0.5% class intervals. It

TABLE IV

STATISTICAL CONSTANTS FOR DATA ON OIL CONTENT, SCALE READING OF HALOWAX-BROMNAPHTHALENE EXTRACT, IODINE VALUE AND SCALE READING OF ETHER EXTRACT

(N = 106)

Means, standard deviations and ranges

		Mean	S.D.	Range
Oil content, %	(x)	40.48	1.84	35.1 – 43.3
Halowax-bromnaphthalene extract scale reading	(y)	20.58	4.57	6.5 – 26.2
Iodine value (Wijs)	(z)	183.79	3.61	179.0 – 193.3
Ether extract scale reading	(s)	41.47	3.57	33.5 – 50.3

Simple correlation coefficients

		r	t*
Oil content × halowax-bromnaphthalene extract scale reading	r_{xy}	.977	46.7
Oil content × iodine value	r_{xz}	−.160	1.6
Halowax-bromnaphthalene extract scale reading × iodine value	r_{yz}	−.129	1.3
Halowax-bromnaphthalene extract scale reading × ether extract scale reading	r_{ys}	.094	1.0
Iodine value × ether extract scale reading	r_{zs}	−.647	8.7

*At 5% pt. t = 1.9

Regression coefficients

		B	t
Oil content on halowax-bromnaphthalene extract scale reading	b_{xy}	.393	46.7
Iodine value on ether extract scale reading	b_{zs}	−.646	8.7

Linear regression equations

	Standard error of prediction
Estimation of oil content from halowax-bromnaphthalene extract scale reading $x = 32.40 + .393\ y$	0.39%
Estimation of iodine value from ether extract scale reading $z = 210.59 - .646\ s$	2.75

will be noted that the mean values for oil content fall very close to the regression line with the exception of two classes for which the frequencies are low. The refractometric scale readings were not converted to refractive indices in making the statistical computations, as in routine practice it would be much more convenient to convert the instrumental readings directly to oil content. However, the refractive indices corresponding to unit scale values are given in the regression graph and also in the tentative conversion chart reproduced in Table V, which was computed from the regression equation $x = 32.40 + .393\ y$.

In regard to the regression of the iodine value of ethyl ether extracts on the refractometric scale readings of the extract an analysis of variance showed that the deviations from regression were not significant. The regression graph is given in Fig. 3 and it will be noted from Table IV that the standard error of prediction of iodine value (Wijs) from the regression equation $z = 210.59 - .646s$ is ± 2.75. In this equation and the conversion chart computed therefrom no cognizance has been taken of the residual oil which may be recovered by re-grinding and re-extraction, since this procedure is not in general use.

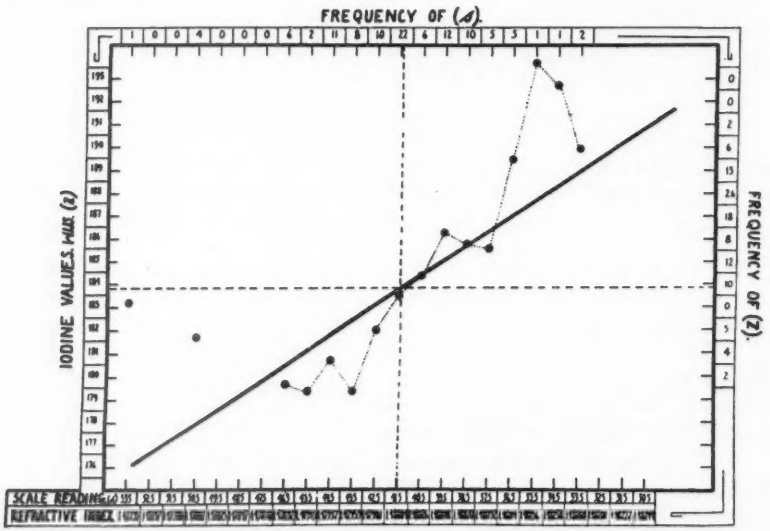

FIG. 3. *Regression of iodine value on refractive index and scale reading of ethyl ether extracts of flaxseed.*

Casual observations indicate that the refractive index of ethyl ether extract of flaxseed is quite sensitive to variations in the temperature at which the ether is distilled off upon completion of extraction and that the changes in refractive index do not appear to be accompanied by corresponding changes in iodine value. While every attempt has been made to standardize the technique in this series, it seems quite possible that a greater degree of accuracy in predicting iodine value from the refractive index of linseed oil would be obtainable in the instance of expressed oils; also the variations noted in the refractive index of ethyl ether extracts of individual samples may, as previously indicated, be responsible for the insignificant influence of the refractive index of the oil upon that of the halogenated naphthalene extracts.

In view of the fact that the halowax and halowax-bromnaphthalene extractions were not conducted on precisely the same samples throughout, and of

TABLE V

TENTATIVE CONVERSION CHART FOR COMPUTING OIL CONTENTS FROM SCALE READING OR REFRACTIVE INDEX OF HALOWAX–α-BROMNAPHTHALENE EXTRACT OF FLAXSEED

Scale reading, (25° C.)	n. (25° C.)	Oil content, %	Scale reading, (25° C.)	n. (25° C.)	Oil content, %	Scale reading, (25° C.)	n. (25° C.)	Oil content, %
7.0	1.62075	35.2	14.2	1.61855	38.0	21.4	1.61633	40.8
7.2	069	35.2	14.4	849	38.0	21.6	626	40.9
7.4	063	35.3	14.6	842	38.1	21.8	620	41.0
7.6	057	35.4	14.8	836	38.2	22.0	614	41.0
7.8	051	35.5	15.0	830	38.3	22.2	608	41.1
8.0	045	35.5	15.2	824	38.4	22.4	602	41.2
8.2	039	35.6	15.4	818	38.4	22.6	595	41.3
8.4	033	35.7	15.6	811	38.5	22.8	589	41.4
8.6	026	35.8	15.8	805	38.6	23.0	583	41.4
8.8	020	35.8	16.0	799	38.7	23.2	577	41.5
9.0	014	35.9	16.2	793	38.8	23.4	571	41.6
9.2	008	36.0	16.4	787	38.8	23.6	564	41.7
9.4	002	36.1	16.6	781	38.9	23.8	558	41.8
9.6	1.61996	36.2	16.8	775	39.0	24.0	552	41.8
9.8	990	36.2	17.0	769	39.1	24.2	546	41.9
10.0	984	36.3	17.2	763	39.2	24.4	539	42.0
10.2	978	36.4	17.4	757	39.2	24.6	533	42.1
10.4	972	36.5	17.6	750	39.3	24.8	526	42.1
10.6	965	36.6	17.8	744	39.4	25.0	520	42.2
10.8	959	36.6	18.0	738	39.5	25.2	514	42.3
11.0	953	36.7	18.2	732	39.6	25.4	508	42.4
11.2	947	36.8	18.4	726	39.6	25.6	501	42.4
11.4	941	36.9	18.6	719	39.7	25.8	495	42.5
11.6	935	37.0	18.8	713	39.8	26.0	489	42.6
11.8	929	37.0	19.0	707	39.9	26.2	483	42.7
12.0	923	37.1	19.2	701	39.9	26.4	477	42.8
12.2	917	37.2	19.4	695	40.0	26.6	470	42.8
12.4	911	37.3	19.6	688	40.1	26.8	464	42.9
12.6	904	37.4	19.8	682	40.2	27.0	458	43.0
12.8	898	37.4	20.0	676	40.2	27.2	452	43.1
13.0	892	37.5	20.2	670	40.3	27.4	446	43.2
13.2	886	37.6	20.4	664	40.4	27.6	439	43.2
13.4	880	37.7	20.6	657	40.5	27.8	433	43.3
13.6	873	37.7	20.8	651	40.6	28.0	427	43.4
13.8	867	37.8	21.0	645	40.6			
14.0	861	37.9	21.2	639	40.7			

Refractive index of halowax–α-bromnaphthalene mixture = 1·64461 at 25° C.

the more satisfactory results secured by the use of the latter solvent, a further series of 100 samples was extracted in duplicate, employing three solvents, namely: halowax, a mixture of approximately 50% by volume of halowax and α-bromnaphthalene, and a 20–80 mixture by volume of halowax and bromnaphthalene.

The relations between oil content and the refractometric readings obtained for the first two solvents agreed closely with those previously reported in detail. However, the degree of association between the means of duplicate scale readings and oil content for the 20–80 halowax-bromnaphthalene extraction was of a lower order of magnitude than for either of the solvents previously employed, and the differences between duplicates were significantly higher.

In view of the excellent agreement obtained between the two series of determinations employing the 50–50 mixture, and also taking into consideration its lower cost, no further investigation was made of the 20–80 mixture to determine the reasons for the apparently anomalous results obtained by its use. It should be mentioned, however, that it was necessary to employ two sets of prisms in order to cover the range in refractive index encountered and this may possibly account in part for the differences found, although the instrument was carefully re-standardized upon changing the prisms.

It will be recalled that, owing to the difficulty with cloudy filtrates when samples contain more than approximately 5% moisture, all samples were dried prior to extraction. This procedure detracts from the rapidity of the test and its value for routine purposes. Experiments were accordingly undertaken to determine whether satisfactory results could be secured by the optical method when anhydrous salts were employed for the removal of moisture. Three anhydrous salts, sodium sulphate, copper sulphate and sodium carbonate, in slight excess, were tried with satisfactory results, the first mentioned appearing to yield slightly clearer filtrates and lower variability between replicates.

TABLE VI

TYPICAL RESULTS OBTAINED BY IMPROVED REFRACTOMETRIC METHOD ON SAMPLES DRIED IN THE VACUUM OVEN *vs.* WITH ANHYDROUS SODIUM SULPHATE

Sample No.	Estimated oil content (50% mixture halowax bromnaphthalene extraction)	
	Dried *in vacuo*, %	Dried with Na_2SO_4, %
1	40.3	40.3
2	40.8	40.9
3	40.2	40.2
4	41.4	41.4
5	40.6	40.6
6	41.6	41.8
7	42.1	42.1
8	42.2	42.2
9	43.4	43.2
10	43.0	43.1
11	41.3	41.4
12	41.1	41.1

A number of samples were tested at their original moisture content, 0.15 gm. Na_2SO_4* being added to the pulp prior to the addition of the 50–50 halowax-bromnaphthalene solvent, and the results compared with those obtained on corresponding samples dried in the vacuum oven prior to extraction. A portion of the data is presented in Table VI, from which it will be noted that excellent agreement was obtained.

Discussion

The results of this study indicate that a mixture of approximately 50% by volume of α-bromnaphthalene and halowax, with a refractive index of 1.64461 at 25° C., offers distinct advantages over halowax as a solvent for linseed oil in the estimation of the oil content of flaxseed by means of the

* *Subsequent to the completion of these experiments, Leithe (Leithe, Wolfgang, Angew, Chem. 47 : 734-736, 1934) has published the details of a refractometric method for the determination of the oil content of oil bearing seeds in which the sample is pulverized with sea sand and anhydrous sodium sulphate prior to extraction with a gasoline fraction (b.p. 90°–100° C.).*

refractometer. The mixed solvent has a higher refractive index than halowax alone and by slight variations in the composition of the mixture the refractive index of the solvent may be adjusted to a constant value, thus rendering it possible to prepare a conversion table for permanent use. The accuracy of estimation of oil content, employing the procedure outlined, may be regarded as fairly satisfactory, especially when the simplicity and rapidity of the method are taken into consideration and also considering the possibility that variations in refractive index of the oil may exert a significant influence on the refractometric readings. While the present study has failed to show such an influence, it will be recalled that the refractive indices were determined on ether-extracted oils and there is some indication that such determinations may be unreliable as a measure of the true refractive index of the linseed oil as extracted by the halogenated naphthalene solvents. Under these circumstances, the conversion chart given in Table V may be regarded as tentative. If the studies now in progress indicate that variations in the refractive index of cold-pressed oil significantly influence the refractometric readings, a correction can be readily applied.

The use of anhydrous sodium sulphate for removal of moisture permits determinations to be made on samples as received without oven drying. The cost of the solvent required for triplicate determinations is approximately $0.063 per sample and the test can be performed with only a limited amount of preliminary training. It would therefore appear that the method would prove of considerable practical utility for routine determinations on large numbers of samples.

References

1. COLEMAN, D. A. and FELLOWS, H. C. U.S. Dept. Agr. Bull. 1471. 1927.
2. COLEMAN, D. A. and FELLOWS, H. C. U.S. Dept. Agr. Tech. Bull. 71. 1928.
3. FISHER, R. A. Statistical methods for research workers, 5th ed. Oliver and Boyd, London. 1934.
4. GEDDES, W. F. and LEHBERG, F. H. Can. J. Research, C, 14 : 45-47. 1936.
5. WESSON, D. Cotton Oil Press 4 : 70-73. 1920.

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STUDIES ON THE ENDOPARASITIC FAUNA OF TRINIDAD MAMMALS

II. ADDITIONAL PARASITES FROM TRINIDAD DEER

BY THOMAS W. M. CAMERON

Abstract

Setaria bidentata (Molin) and *Eucyathostomum longesubulatum* Molin, are recorded from *Mazama simplicicornis* and are re-described together with a species of Oxyurid from the same host.

In a previous article in this Journal (1) a number of helminth parasites from a deer from Trinidad were described. Thanks to the co-operation of Professor Urich, the entrails of a second deer of the same species *(Mazama simplicicornis)* have recently been received from Trinidad and a careful examination has revealed the presence of three species of nematodes. Two of these appear to be old forms described briefly by Molin in 1858 and 1861 from deer in Brazil and not since seen, while the third appears to be unrecorded.

Setaria bidentata (MOLIN, 1858) RAILLIET ET HENRY, 1911

Two males and three females were recovered from the omentum in a good state of preservation. The cuticle is longitudinally striated with many fine lines which, however, are absent from the lateral areas of the body. The head is rounded and not separate from the remainder of the body. The peribuccal ring is prominent (Figs. 1–4) but is notched only laterally to give the appearance of single dorso-ventral teeth. Each tooth is complete and is *not* indented. The peribuccal ring is surrounded by the normal six circum-oral papillae. The appearance is similar in both sexes, although the ring is more conspicuous in the female than in the male.

The females have an average length of 75 mm. and a maximum thickness of 0.8 mm. The oesophagus is divided into the usual two parts; the anterior, more muscular portion being about 0.56 mm. long, the posterior more glandular portion, about 4.0 mm. long. The nerve ring surrounds the anterior portion at a point about a third of its length from the mouth opening, while the cervical papillae, which are small and inconspicuous, are slightly posterior to this. A number of strands of tissue leave the nerve ring and proceed to the cuticle where they are connected to depressed areas (Fig. 1) which are

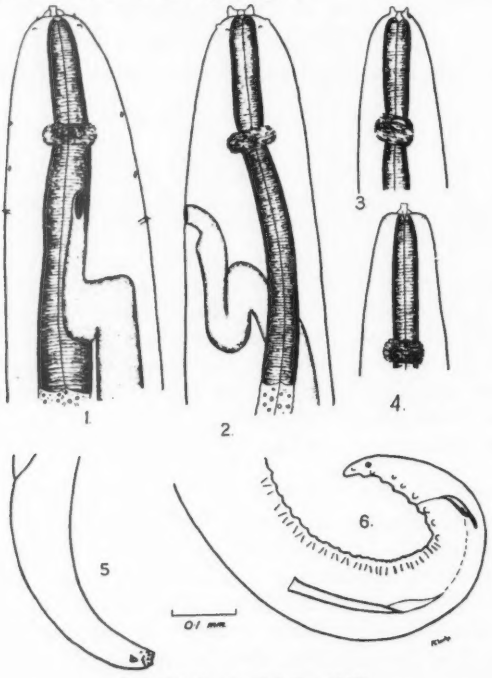
[1] *Manuscript received December 16, 1935.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Quebec, with financial assistance from the National Research Council of Canada.

[2] *Research Professor of Parasitology, McGill University; and Director, Institute of Parasitology, Macdonald College, P.Q., Canada.*

possibly of a sensory nature. The female genital aperture is found close to these areas and they may function in connection with it.

The genital system is of the typical *Setaria* type.

The tail is slender and curved dorsally. It is 0.4 mm. long and terminates in a knob-like process covered with blunt spines. Two lateral, pointed appendices are found close to the tip (Fig. 5).



Setaria bidentata (Molin, 1858)

FIG. 1. *Head of female, ventral view.*
FIG. 2. *Head of female, lateral view.*
FIG. 3. *Head of male, lateral view.*
FIG. 4. *Head of male, ventral view.*
FIG. 5. *Tail of female.*
FIG. 6. *Tail of male.*

The male is smaller than the female and the tail end more tightly coiled. The two examples seen measured 33.75 mm. and 35.5 mm. respectively; the maximum thickness was 0.5 mm. The head end is similar to that seen in the female. The total length of the oesophagus is 0.35 mm.

The tail is bluntly pointed. There are four pairs of ventral post-anal papillae and a single pair of lateral post-anal papillae close to the tip. Only three pairs of pre-anal papillae were observed. From just anterior of the ano-genital opening to a point about 4.25 mm. from the tip of the tail, there are numerous *ventral* thickenings of the body, which under a low power of the microscope, superficially resemble papillae. The cuticle between these is transversely striated ventrally.

The two spicules are very dissimilar (Fig. 6). The smaller one is about 0.075 mm. long. The larger one is partly tubular and partly filamentous, the tubular part measuring 0.18 mm. long.

Three species of *Setaria* are known to occur in South American ruminants: *Setaria labiato-papillosa* (in cattle), *Setaria nudicauda* Ortlepp, 1924, from an unidentified member of the Cervidae in Dutch Guiana, and *Setaria bidentata* (Molin, 1858) from *Mazama nana* from Brazil. The present species differs from *S. labiato-papillosa* (which is practically cosmopolitan in its distribution) in a variety of points, especially in the shape of the peribuccal ring, and from *S. nudicauda*, in possessing a number of spines on the tail of the female.

Molin recorded *Filaria bidentata* from a closely related host in 1858. He described it as having the mouth armed with two teeth, both ends of the body tapering, the male tail rolled into many spirals, provided with numerous papillae ventrally and definitely pointed; the female tail loosely spiralled, its tip mucronate and spinose. The male measured up to $1\frac{1}{4}$ in. (32 mm.) and the female from $1\frac{3}{4}$ to $2\frac{3}{4}$ in. (44 to 70 mm.) in length.

This parasite does not appear to have been subsequently described. The original description is inadequate by modern standards but there is nothing in it which is incompatible with the description of the present species. Molin's reference to the numerous ventral papillae can quite well be interpreted as a reference to the true ventral papillae, together with the numerous ventral cuticular thickenings, which superficially resemble papillae. Moreover the hosts are very closely related zoologically. Accordingly, it is believed that the present species is the same as that seen in 1858 by Molin.

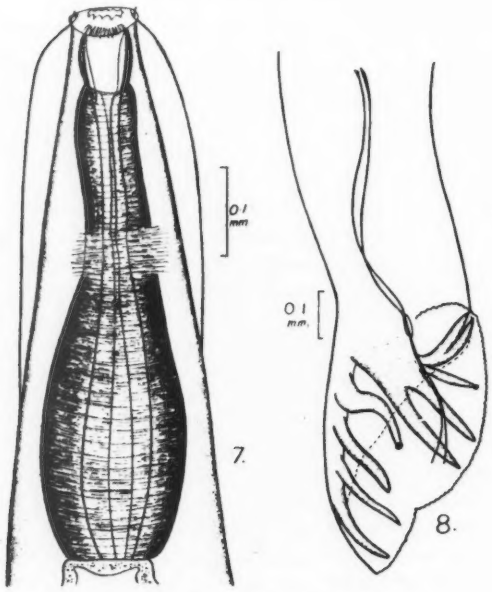
Eucyathostomum longesubulatum MOLIN, 1861

This parasite was recorded and described by Molin in 1861 from the caecum of the Pampas Deer *(Blastocerus campestris)* and the Red Brocket *(Mazama rufa)* from Brazil, but it does not appear to have been seen since. The writer recovered two males from the large intestine of *Mazama simplicicornis*.

The two males measure 10.5 mm. and 11.25 mm. respectively in length and 0.32 mm. in thickness. The cuticle is finely striated laterally with numerous longitudinal striations in the anterior region.

The mouth opening is terminal (Fig. 7) and communicates with a large, almost cylindrical buccal capsule, the inner surface of which carries a number of ridges, but no teeth. The writer was unable to identify a dorsal gutter. An internal leaf crown of some 28 pointed elements arises from the anterior edge of the buccal capsule. The mouth is surrounded by a ring of granular cuticle which is crenulated around the actual opening; this ring carries six circum-oral papillae.

The oesophagus is of the usual strongyle type and is 0.55 mm. long. The nerve ring is just anterior to the middle, but the small cervical papillae are situated almost at the level of the base of the oesophagus. The cuticle of the head region is swollen and the excretory pore is situated just at the posterior edge of this swelling.



Eucyathostomum longesubulatum Molin, 1861

FIG. 7. *Head of male.*

FIG. 8. *Bursa of male, lateral view.*

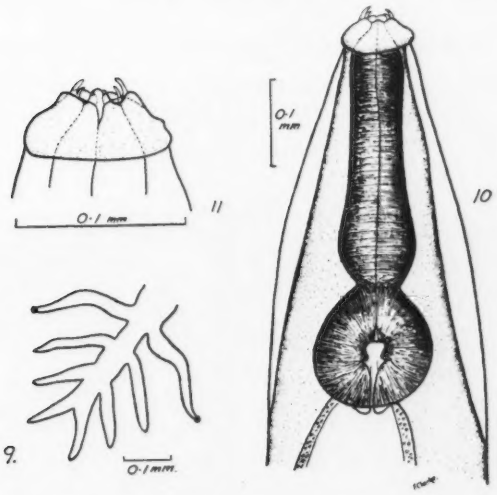
The genital system is of the usual strongyle type.

The bursa (Fig. 8) is voluminous and has a large dorsal lobe. The lateral and ventral rays arise from a common trunk. The ventral rays lie close together and are situated at right angles to the lateral rays. The lateral rays diverge slightly from each other, the externo-lateral being smaller and more slender than the others.

The dorsal ray is large and massive, the externo-dorsals arising some distance down its main stem. It is bifurcated distally (Fig. 9), each bifurcation being massive and simple. Two external branches are given off on each side of the dorsal ray between the externo-dorsal and the termino-dorsal rays.

The spicules are equal, long and filiform, measuring 0.9 mm. in length. Their free ends have a single lateral ala. Their points are barbed but are not fused together as in *Necator*. A small transparent accessory piece is present.

No females were found, but Molin describes these as 15 to 20 mm. long, with a long pointed tail. The vulva is situated just in front of the anus and has a distinct superior lip.



Eucyathostomum longesubulatum Molin, 1861.
FIG. 9. *Dorsal lobe of bursa.*

"Oxyuris" (s. l.) sp.
FIG. 10. *Head and oesophagus.*
FIG. 11. *Mouth opening.*

"Oxyuris" (s. l.) sp.

A single female oxyurid worm was also present in the large intestine, but owing to its immature condition and the absence of males, it is impossible to refer it to a genus.

It is 6.75 mm. long and has a maximum thickness of 0.3 mm. The cuticle is free from striations but the posterior end—especially the portion posterior to the anus—carries a number of small annular bands.

The mouth is surround by a granular cuticular collar (Figs. 10 and 11) carrying three fleshy lips and three small interlabia. Each lip has a long, transparent, medially curved process, medial to which is a smaller similar process. The interlabia are slightly trifurcated. The oesophagus is of the usual oxyurid type (Fig. 10), the anterior portion being 0.3 mm. long and the bulb 0.15 mm. in diameter. The cuticle in the oesophageal region is swollen. The excretory pore is situated posterior to the end of the oesophagus, 0.8 mm. from the anterior end of the body.

The vulva is at the junction of the middle and posterior thirds of the body, and the anus is 0.65 mm. from the tip of the sharply pointed tail.

References

1. CAMERON, T. W. M. Studies on the endoparasitic fauna of Trinidad mammals. I. Some parasites of Trinidad deer. Can. J. Research, D, 13 : 89-96. 1935.
2. I.B.A.P. The helminth parasites of deer. Notes and Memoranda, No. 4, p. 32. 1931.

SCHISTOSOME DERMATITIS IN CANADA

NOTES ON TWO CAUSATIVE AGENTS AND THEIR SNAIL HOSTS IN MANITOBA[1]

By W. E. Swales[2]

Abstract

An investigation of "swimmers' itch" at Clear Lake, Manitoba, revealed the presence of two schistosome cercariae, *Cercaria elvae* Miller, 1923 in *Lymnaea stagnalis jugularis* Say, and *Cercaria* sp. in *Stagnicola emarginata canadensis* (Sowerby); both species are known causes of the dermatitis. Five species of strigeid cercariae were found, none of which indicated any power of penetration into human skin. Copper sulphate, appropriately applied, and hand collection of adult snail hosts, are suggested for controlling schistosome dermatitis in Clear Lake.

"Swimmers' itch" or "slough itch" was first recognized as being caused by certain trematode cercariae by Cort (6) in Michigan. Since that time a certain amount of work has been done on the problem by workers in both Europe and North America. Several species of cercariae have been incriminated as causative agents, and until the work of McLeod (11) appeared they were all shown to be apharyngeal brevifurcate distome cercariae or typical members of the family Schistosomidae. However, McLeod recorded three species of cercariae from Clear Lake, Manitoba, all of which he described as penetrating human skin and causing a dermatitis. Two of these species he described as new to science and according to the illustrations they are members of the family Strigeidae and not Schistosomidae. The third is *Cercaria elvae* Miller, 1923, from a new snail host, *Lymnaea obrussa*.

In response to a request made by the National Parks Branch, Department of the Interior, the writer investigated the occurrence of the dermatitis at Clear Lake, Manitoba, in August, 1935. The following observations were made at Clear Lake and also upon material collected there and returned to the University of Michigan Biological Station and this Institute.

A survey of the beaches upon which the dermatitis has been recorded revealed the presence of a limited snail fauna as follows:—

Stagnicola emarginata canadensis (Sowerby) (Figs. 3–6); *Lymnaea stagnalis jugularis* Say. (Figs. 1–2); *Helisoma trivolvis* (Say); *Helisoma* sp.; *Helisoma* sp.; *Aplexa hypnorum tryoni* Currier; *Stagnicola palustris* var.; *Physella gyrina* (Say).

On the sandy beaches, which were almost devoid of macroscopic vegetation, the only snail in evidence was *S. emarginata canadensis*. This species was very common on the sand and stones at from three to seven feet below the surface

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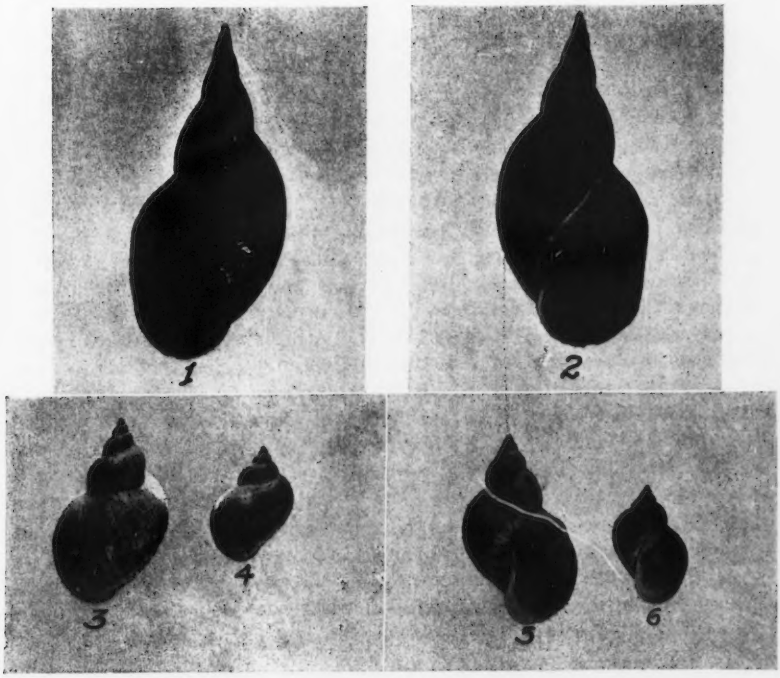
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Macdonald College Post Office, Quebec; with financial assistance from the National Research Council of Canada. Also partly contributed from the Biological Station, University of Michigan.
[2] *Research Assistant.*

PLATE I



FIGS. 1–2. *Lymnaea stagnalis jugularis Say, from Clear Lake, Manitoba. (Actual length—4 cm.)*

FIGS 3–6. *Stagnicola emarginata canadensis (Sowerby) from Clear Lake, Man. (Actual lengths—2.3 cm. and 1.4 cm.)*

of the water. In two small adjoining bays and in a creek (now dammed from the lake), which contained an abundance of macroscopic vegetation, the fauna included:—

L. stagnalis jugularis, *S. palustris* var., *A. hypnorum tryoni*, *P. gyrina*, and the *Helisoma* spp.

The bays also appeared to be the breeding places of *S. emarginata canadensis*, numerous young specimens of this species being present on the vegetation in shallow water.

Each snail collected was isolated in clear tap water and observed frequently over a period of 48 hours. If at the end of this period no cercariae had emerged, the specimen was crushed and examined microscopically. The results of these observations were noted and all infested snails which had shed cercariae were later taken alive to the Biological Station for further study of the cercariae. Still later, many specimens were collected from the beaches by co-operators and submitted to this Institute for further records.

The results of the field tests are as follows: Of 120 *S. emarginata canadensis* collected from the bathing beaches, seven shed schistosome cercariae alone, 24 shed strigeid cercariae alone, two shed both schistosome and strigeid cercariae, and one shed stylet cercariae. Of 272 specimens of *L. stagnalis jugularis*, collected from the creek and from one of the bays, 15 shed schistosome cercariae, 11 shed strigeid cercariae, and two shed both types. Of the other snails collected, *Helisoma trivolvis* was infested, two specimens of this species shedding a small straight-tailed strigeid, *Cercaria burti* Miller, 1923, in large numbers.

Almost every *S. palustris* var. tested shed echinostome cercariae or stylet cercariae, but no furcocercous forms.

Following a field study of the cercariae and of the ecology of the snail hosts, the living parasitized snails were taken to the University of Michigan Biological Station. The cercariae were there studied and comparisons were made with specimens from type or adjoining localities in Michigan.

The schistosome cercaria from *S. emarginata canadensis* was found to be identical with a schistosome cercaria from *S. emarginata angulata*. Dr. Benton S. Talbot, working at Douglas Lake, had recognized this species as being new to science and his description will be published in the near future. It must be referred to here as *Cercaria* sp. Twenty-one specimens of the former snail shed a single species of cercaria—that of *Diplostomum flexicaudum* (Cort and Brooks, 1928). The other three each shed a single species of strigeid cercariae, two having been previously recorded at Douglas Lake by Professor Cort, but as yet undescribed. The other was a strigeid of the Alaria type.

The two mixed infestations revealed the schistosome *Cercaria* sp. and *D. flexicaudum*.

The schistosome cercariae from *L. stagnalis jugularis* were all of a single species, *Cercaria elvae* Miller, 1923. All the strigeid cercariae found in this snail were also of a single species, *Diplostomum flexicaudum*.

In addition to the above, several specimens of *S. emarginata canadensis* contained tetracotyles of *Cotylurus flabelliformis*.

The snail fauna and the cercariae of Clear Lake do not differ materially from those of several lakes in the vicinity of the University Biological Station, Michigan. Differences noted were the scarcity of Physidae at Clear Lake and the apparent limitation of the dermatitis-producing schistosome cercariae to two species.

Owing to the fact that two species of strigeid cercariae had previously been reported as causing a dermatitis, each species of furcocercous cercaria collected was tested to determine its power of penetrating human skin. Three susceptible volunteers were first used, and later four more were used for comparison. The cercariae were carefully isolated under a binocular microscope to avoid confusion due to mixed infestations.

In every case *Cercaria elvae* from *L. stagnalis jugularis* caused lesions typical of schistosome dermatitis. In four subjects the schistosome *Cercaria* sp. from *S. emarginata canadensis* caused similar lesions. The writer is very susceptible to the dermatitis and the sensations experienced on several experimental exposures were similar to those described by other workers. As the water containing the cercariae dries on the skin a prickling sensation is felt. This subsides in from five to ten minutes, leaving no visible lesion. During the night following the day of exposure an intense skin irritation occurs and the next day red papules, 5–15 mm. in diameter, are present. In my case these papules, unless scratched, disappeared by the third day, and the irritation was noticed only during the first 24 hours after exposure. No cercariae other than those mentioned gave any indication of penetrating skin, even although they were placed on arms and legs in large numbers and the water was allowed to evaporate.

Discussion of the Observations

Clear Lake, in common with similar lakes in the mid-western United States, has been found to be a habitat of two important gastropods, varieties of *S. emarginata* and *L. stagnalis*. Both of these snails are intermediate hosts of schistosome trematodes, the cercariae of which are capable of penetrating human skin and causing a temporary dermatitis. Owing to the lack of macroscopic vegetation and the sandy and stony bottoms of the beaches, *S. emarginata* is the common snail on the areas used for bathing by visitors. *L. stagnalis* is limited in its distribution near the beaches to small bays and a creek which contain an abundance of vegetation. Thus *Cercaria elvae* is of secondary importance as a causative agent of dermatitis, whereas the schistosome *Cercaria* sp. from *S. emarginata* is actually present on the bathing beaches whenever conditions are favorable for its emergence. Of the two snail hosts *L. stagnalis jugularis* can be destroyed by means of copper sulphate broadcast over the shallow water habitats. In controlling *S. emarginata canadensis* the methods must be such that the lethal agent will diffuse over the surface of the sand and stones in deeper water. Collection by hand of the adult snails is also indicated as a means of reducing their numbers.

The most common cercaria in Clear Lake, that of *D. flexicaudum*, is a strigeid, and does not cause the dermatitis. It is, however, very pathogenic to fish, penetrating the eyes and causing intense inflammatory processes, which, in tests conducted with Clear Lake material, were followed by death in 48 to 72 hours. Young *Ameiurus nebulosus* were found to have the cercariae actively moving around the lens of the eyes after the fish's death. As pointed out by Van Haitsma (16) and others, this cercaria might have an important bearing on the health of fresh-water fishes.

The cercaria of *D. flexicaudum* is the strigeid found in mixed infestations with schistosome cercariae, in both snail hosts in Clear Lake. This fact might lead to confusion in certain cases.

The writer's findings must be compared with those of McLeod who worked on the same area in 1933. McLeod (11) found one schistosome and two strigeid species in *Lymnaea obrussa*. This snail (now *Fossaria obrussa* (Say) Baker, 1928) according to Baker (1, 2) is found generally in small bodies of water, such as creeks, ponds, sloughs, bays and marshy spots along river banks. It frequently inhabits abandoned quarries which have become filled with water. Like *Fossaria modicella* and *F. parva*, this species is at home on sticks, stones and exposed muddy surfaces. Although the writer has frequently collected *F. obrussa* in other parts of Canada he was unable to find it on the shore of Clear Lake. It seems possible that McLeod was working with *Stagnicola emarginata canadensis*. In any case the occurrence of *Cercaria elvae* in either of these snails needs further study.

Cercaria bajkovi McLeod, 1934, was not found during this investigation but a continued search might have revealed this species. In the search for *C. wardlei* McLeod, 1934, each lot of the peculiar flexed-tail forms was carefully studied, without result. In each case these forms were the cercariae of *D. flexicaudum*, which according to McLeod (11) differ from *C. wardlei* in the number of penetration glands, the caudal flame-cell pattern and in the absence of oral bristles in the latter species.

Owing to the great prevalence of strigeid cercariae in all fresh water, McLeod's record of the penetration of mammalian skin by members of this group is important and needs further study.

Acknowledgments

I wish to express great appreciation of the help and co-operation of the National Parks Branch of the Department of the Interior of Canada. Mr. J. Smart, Park Superintendent, who laid the foundation for investigational work on this subject at Clear Lake, and who is now applying measures of control, provided invaluable assistance. Dr. G. Stevens, Parks Physician, also helped materially.

To Drs. Geo. LaRue, W. W. Cort and Benton Talbot, I am deeply indebted for the opportunity to work at Douglas Lake and for invaluable advice, direction, and confirmation of identifications. Mr. A. La Rocque, Department of Mines, Ottawa, kindly identified the snail hosts.

References

1. BAKER, F. C. The Lymnaeidae of North and Middle America. Chicago Acad. Sci., Spec. Pub. No. 3. 1911.
2. BAKER, F. C. The ecology of North American Lymnaeidae. Science, n.s., 49 : 519-521. 1919.
3. BAKER, F. C. The Fresh Water Mollusca of Wisconsin. Part I. Gastropoda. Wisc. Geol. Nat. Hist. Survey, Bull. 70. 1928.
4. BRUMPT, E. Prurit et dermatitis produits chez les nageurs par des cercaires des mollusques d'eau douce. Compt. Rend. Acad. Sci. 193 : 253-255.
5. CHRISTENSON, R. O. and GREEN, W. P. Studies on biological and medical aspects of "swimmers' itch." Minn. Med. 11 : 573-575. 1928.
6. CORT, W. W. Schistosome dermatitis in the United States. J. Am. Med. Assocn. 90 : 1027-1029. 1928.
7. CORT, W. W. Further observations on schistosome dermatitis in the United States (Michigan). Science, 68 : 388. 1928.
8. CORT, W. W. and BROOKS, S. T. Studies on the Holostome Cercariae from Douglas Lake, Michigan. Trans. Am. Micros. Soc. 47 : 179-221. 1928.
9. LA RUE, G. R. Studies on the trematode family Strigeidae (Holostomidae) No. III. Relationships. Trans. Am. Micros. Soc. 65 : 265-281. 1926.
10. LA RUE, G. R., BUTLER, E. P. and BERKHOUT, P. F. Studies . . . Strigeidae. No. IV. The eye of fishes, an important habitat for larval Strigeidae. Trans. Am. Micros. Soc. 65 : 282-288. 1926.
11. MCLEOD, L. A. Notes on cercarial dermatitis with descriptions of the caustive organisms, *Cercaria wardlei* n. sp., *Cercaria bajkovi* n. sp., and the parthenogenic stage of *Cercaria elvae* Miller. Can. J. Research, 10 : 394-403. 1934.
12. MILLER, H. M. Comparative studies on furcocercous cercariae. Illinois Biol. Mon., 10 : No. 3 1-112. 1926.
13. PRICE, E. W. A synopsis of the trematode family Schistosomidae with descriptions of new genera and species. Proc. U.S. Nat. Mus. 75 : Art. 18. 1-39. 1929.
14. SZIDAT, L. and WIGAND, R. Leitfaden der einheimischen Wurmkrankheiten des Menschen. pp. 1-212. George Thieme, Verlag, Leipzig. 1934.
15. TAYLOR, E. L. and BAYLIS, H. A. Observations and experiments on a dermatitis-producing cercaria and on another cercaria from *Limnaea stagnalis* in Great Britain. Trans. Roy. Soc. Trop. Med. Hyg. 24. 219-244. 1930.
16. VAN HAITSMA, J. P. Studies on the trematode family Strigeidae (Holostomidae). No. xxiii. *Diplostomum flexicaudum* (Cort and Brooks) and stages in its life-history. Papers Mich. Acad. Sci. Arts. and Letters. 13 : 483-513. 1931.
17. VOGEL, H. Cercarien-Dermatitis in Deutschland. Klin. Wochschr. 9th yr. 883-886. 1930.